Assessment of Small Airway Function
– Application of Impulse Oscillometry in Young Children with Asthmatic Symptoms

Hanna Knihtilä, MD

Academic Dissertation
Doctoral Programme in Clinical Research

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in the auditorium of the Skin and Allergy Hospital, Helsinki University Hospital, on Friday, November 30th, 2018, at 12 noon.

Helsinki 2018
To my family
“A winner is a dreamer who never gives up.” – Nelson Mandela
TABLE OF CONTENTS

ORIGINAL PUBLICATIONS ........................................................................................................... 7
ABBREVIATIONS .......................................................................................................................... 8
ABSTRACT ...................................................................................................................................... 10
TIIVISTELMÄ (ABSTRACT IN FINNISH) .................................................................................... 11
1 INTRODUCTION ...................................................................................................................... 12
2 REVIEW OF THE LITERATURE .............................................................................................. 13
   2.1 Development and anatomy of the respiratory system ......................................................... 13
   2.2 Obstructive respiratory symptoms in childhood ................................................................. 15
      2.2.1 Symptoms suggestive of asthma ................................................................................... 15
         2.2.1.1 Pathophysiology ...................................................................................................... 15
         2.2.1.2 Epidemiology and morbidity .................................................................................... 17
         2.2.1.3 Phenotypes ............................................................................................................... 17
         2.2.1.4 Clinical assessment .................................................................................................. 19
      2.2.2 Bronchopulmonary dysplasia ......................................................................................... 21
   2.3 Assessment of small airway function and inflammation .................................................... 23
      2.3.1 Impulse oscillometry ...................................................................................................... 25
         2.3.1.1 Technical principles and parameters ....................................................................... 25
         2.3.1.2 Performing the measurement and assessing reliability ......................................... 28
         2.3.1.3 Assessing bronchial reversibility and hyperreactivity ............................................ 29
      2.3.2 Spirometry ...................................................................................................................... 31
      2.3.3 Multiple breath washout tests ....................................................................................... 33
      2.3.4 Extended exhaled nitric oxide measurements ............................................................... 35
   2.4 Small airways in respiratory disease .................................................................................. 37
   2.5 Tracking of lung function .................................................................................................... 39
3 OBJECTIVES ........................................................................................................................... 41
4 METHODS ................................................................................................................................. 42
   4.1 Study subjects and designs ................................................................................................. 42
   4.2 Measurements of lung function and airway inflammation ................................................ 46
   4.3 Atopy and eosinophilia ...................................................................................................... 48
   4.4 Statistical analyses .............................................................................................................. 48
4.5 Ethics ................................................................................................................................................ 50

5 RESULTS .................................................................................................................................................... 51
  5.1 Reference values for R5-20, R5-20%, and AX .................................................................................. 52
  5.2 Sensitivity of IOS in detecting symptomatic children ................................................................. 54
  5.3 Bronchodilator responses of R5-20, R5-20%, and AX in healthy children ............................... 56
  5.4 Repeatability of IOS in symptomatic children ........................................................................... 58
  5.5 Small airway IOS, spirometry, MBNW, and FeNO indices ......................................................... 59
      5.5.1 Differences between healthy and symptomatic children ................................................... 59
      5.5.2 Association with asthma control and AHR ..................................................................... 62
      5.5.3 Association between the small airway IOS indices and other techniques ................. 63
  5.6 Association between preschool IOS and spirometry in adolescence .................................... 64

6 DISCUSSION ........................................................................................................................................... 67
  6.1 Reference values and repeatability of R5-20, R5-20%, and AX ............................................. 67
  6.2 Small airway abnormalities in different patient groups .......................................................... 68
  6.3 Association of small airway function with asthma control and AHR .................................... 70
  6.4 Small airway dysfunction at preschool age and later lung function ..................................... 70
  6.5 Study limitations .......................................................................................................................... 71
  6.6 Clinical implications and future considerations ..................................................................... 72

7 CONCLUSIONS ..................................................................................................................................... 74

ACKNOWLEDGEMENTS ................................................................................................................................. 75

REFERENCES .................................................................................................................................................. 77
This thesis is based on the following publications:


The publications are referred to in the text by their Roman numerals. They have been reprinted with the permission of their copyright holders. In addition, some unpublished material is presented.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHR</td>
<td>Airway hyperresponsiveness</td>
</tr>
<tr>
<td>ALSPAC</td>
<td>Avon Longitudinal Study of Parents and Children</td>
</tr>
<tr>
<td>API</td>
<td>Asthma Predictive Index</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>AX</td>
<td>Area under the respiratory reactance curve</td>
</tr>
<tr>
<td>BAMSE</td>
<td>Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology</td>
</tr>
<tr>
<td>BDR</td>
<td>Bronchodilator responsiveness</td>
</tr>
<tr>
<td>BPD</td>
<td>Bronchopulmonary dysplasia</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>C\text{\textsubscript{ALV}}</td>
<td>Alveolar nitric oxide concentration</td>
</tr>
<tr>
<td>CAMP</td>
<td>Childhood Asthma Management Program</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>COPSAC</td>
<td>Copenhagen Prospective Studies on Asthma in Childhood</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>C-ACT</td>
<td>Childhood Asthma Control Test</td>
</tr>
<tr>
<td>dR/df</td>
<td>Frequency-dependence of the respiratory resistance</td>
</tr>
<tr>
<td>EIB</td>
<td>Exercise-induced bronchoconstriction</td>
</tr>
<tr>
<td>ERS</td>
<td>European Respiratory Society</td>
</tr>
<tr>
<td>EW</td>
<td>Early wheezing</td>
</tr>
<tr>
<td>FEF\textsubscript{25-75}</td>
<td>Mean expiratory flow between 25% and 75% of forced vital capacity</td>
</tr>
<tr>
<td>FEF\textsubscript{50}</td>
<td>Forced expiratory flow at 50% of forced vital capacity</td>
</tr>
<tr>
<td>FeNO</td>
<td>Fractional concentration of exhaled nitric oxide</td>
</tr>
<tr>
<td>FeNO\textsubscript{50}</td>
<td>Fractional concentration of exhaled nitric oxide at the exhalation flow rate of 50 ml/s</td>
</tr>
<tr>
<td>FEV\textsubscript{1}</td>
<td>Forced expiratory volume in one second</td>
</tr>
<tr>
<td>FOT</td>
<td>Forced oscillation technique</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>Fres</td>
<td>Resonance frequency</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GSDMB</td>
<td>Gasdermin B gene</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticosteroid</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IOS</td>
<td>Impulse oscillometry</td>
</tr>
<tr>
<td>ISAAC</td>
<td>International Study of Asthma and Allergies in Childhood</td>
</tr>
<tr>
<td>J\textsubscript{NO}</td>
<td>Bronchial exhaled nitric oxide flux</td>
</tr>
<tr>
<td>LCI</td>
<td>Lung clearance index</td>
</tr>
<tr>
<td>LR+</td>
<td>Positive likelihood ratio</td>
</tr>
<tr>
<td>LR-</td>
<td>Negative likelihood ratio</td>
</tr>
<tr>
<td>MAAS</td>
<td>Manchester Asthma and Allergy Study</td>
</tr>
<tr>
<td>mAPI</td>
<td>Modified Asthma Predictive Index</td>
</tr>
<tr>
<td>MBNW</td>
<td>Multiple breath nitrogen washout test</td>
</tr>
<tr>
<td>MBW</td>
<td>Multiple breath washout test</td>
</tr>
</tbody>
</table>
N$_2$  Nitrogen gas
NO   Nitric oxide
ORMDL3 Orosomucoid 1-like 3 gene
PEF  Peak expiratory flow
ppb  Parts per billion
$r^2$  Coefficient of determination
R5   Resistance of the respiratory system at 5 Hz
R20  Resistance of the respiratory system at 20 Hz
R5-20 Difference between respiratory resistance at 5 and 20 Hz
R5-20% Relative difference between respiratory resistance at 5 and 20 Hz
ROC  Receiver operating characteristic
RSD  Residual standard deviation
SABA Short-acting beta$_2$-receptor agonist
Sacin Index of ventilation inhomogeneity of the acinar lung zone
Scond Index of ventilation inhomogeneity of the conducting airways
SD  Standard deviation
SDw  Within-subject standard deviation
SnIII Concentration-normalized phase III slope
SPT  Skin prick test
T$_{h2}$ T helper type 2 cell
TLS  Troublesome lung symptoms
$V_{\text{maxFRC}}$ Maximal expiratory flow at functional residual capacity
X5   Reactance of the respiratory system at 5 Hz
X10  Reactance of the respiratory system at 10 Hz
ABSTRACT

Small airway dysfunction has been associated with different clinical features of asthma, such as symptom severity, exacerbations, and airway hyperresponsiveness (AHR). However, the diagnosis and monitoring of asthma focus on indices that reflect mainly large airway function, and no gold standard measure for small airways exists. Especially in children, the clinical significance of small airway dysfunction in asthma is poorly understood. This thesis investigates small airway dysfunction in children with symptoms suggestive of asthma, with a special interest in the clinical utility of impulse oscillometry (IOS) indices postulated to reflect small airway function in these children.

First, IOS results of 103 healthy Finnish children (aged 2-7 years) were used to establish reference values for IOS indices postulated to reflect small airway properties (R5-20, R5-20%, and AX). The ability of these indices to distinguish different phenotypes of early respiratory morbidity was evaluated in 65 symptomatic children (aged 3-8 years). Second, the cut-off values for normal bronchodilator responses (BDR) and between-visit repeatability were determined for these indices. Third, the presence of small airway dysfunction in 58 children (aged 5-10 years) with mild to moderate respiratory symptoms suggestive of asthma was evaluated by comparing their IOS, spirometry, multiple breath nitrogen washout, and alveolar nitric oxide concentration (C_{ALV}) results with those of 19 healthy controls (aged 5-10 years). The associations of small airway dysfunction with asthma control and AHR were also evaluated. Fourth, the longitudinal association between preschool (2-7 years) IOS and spirometry in adolescence (12-18 years) was evaluated in 154 subjects with asthma.

Height- and sex-adjusted reference equations were created for R5-20, R5-20%, and AX. When using the established reference equations, these indices were superior to conventional IOS parameters (R5 and X5) in distinguishing all patient groups from healthy controls. In the healthy reference sample, these indices showed marked BDRs. Although these indices showed larger variability relative to R5, their between-visit agreement remained good in symptomatic children. The proposed small airway indices of IOS, spirometry, and C_{ALV} differed between children with mild to moderate recurrent wheezing and healthy controls. Furthermore, ventilation inhomogeneity indices and C_{ALV} were associated with frequent asthma exacerbations and AHR. In the longitudinal study, preschool IOS parameters, especially those related to small airway function, were associated with lung function in adolescence.

Children with respiratory symptoms suggestive of asthma may present with early changes related to small airway dysfunction and IOS seems to be useful in detecting these children. Furthermore, the proposed small airway IOS indices appear to be associated with later lung function. Although these indices show greater variability than conventional IOS parameters, their between-visit agreement remains good. Therefore, these IOS indices provide a potential tool for evaluating early disease changes and the effects of interventions in young children with symptoms suggestive of asthma.
Pienten hengitysteiden toimintahäiriö on liitetty astman kliiniisiin piirteisiin, kuten oireiden vaikeusasteeseen ja hengitysteiden hyperreaktiviteettiin (AHR). Tavanomaiset keuhkojen toiminnan mittausmenetelmät kuitenkin kuvastavat lähinnä suurempan hengitysteiden toimintaa, ja erityisesti lasten astmassa pienten hengitysteiden merkityksestä on hyvin vähän tutkimustietoa. Väittöskirjatutkimuksen tarkoituksena oli selvittää pienten hengitysteiden toimintahäiriön merkitystä lasten astmaan viittaavissa oireissa ja erityisesti impulssioskilometri-in (IOS) käyttökelpoisuutta näiden muutosten arvioinnissa.

Ensimmäisessä osatyössä määritettiin terveille lapsille (ikä 2-7 vuotta, n=103) viitearvot pienten hengitysteiden toimintaan liitetyille IOS-parametreille (R5-20, R5-20% ja AX). Poikkeavien tulosten yleisyttä tutkittiin lapsilla (ikä 3-8 vuotta, n=65), joilla oli astmaan viittaavia oireita tai bronkopulmonaalinen dysplasia. Toisessa osatyössä määritettiin raja-arvot keuhkoputkia avaavan lääkeaineen aiheuttamille muutoksille näissä parametreissa terveillä lapsilla. Lisäksi arvioitiin IOS:n toistettavuutta astmaoireisilla lapsilla (n=43). Kolmannessa osatyössä tutkittiin pienten hengitysteiden toimintaan liitetyjen IOS-, spirometria-, monihengitysnitrogria- (MBNW) ja uloshengitysilman typpioksidiparametrien (C_{ALV}) kykyä tunnistaa astmaoireiset lapset (ikä 5-10 vuotta, n=58) terveistä verrokeista (ikä 5-10 vuotta, n=19). Neljännässä osatyössä selvitettiin leikki-iän (2-7 vuotta) IOS:n ja teini-iän (12-18 vuotta) spirometriatulosten yhteyttä astmapotilailailla (n=154).


Asthmaoireisten lasten pienten hengitysteiden toiminnassa voidaan havaita eroja terveisiin kontrolloihin verrattuna ja IOS vaikuttaa käyttökelpoiselta näiden muutosten arvioinnissa. Astmapotilaila pienten hengitysteiden toimintahäiriö voi myös olla yhteydessä myöhempään keuhkojen toimintaan. Perinteiä parametra suuremmasta vaihtelusta huolimatta pienten hengitysteiden toimintaan liitetyjen IOS-parametrien 7-14 päivän yhtenevääsyys säilyy hyvänä. IOS tarjoaa potentiaalisen menetelmän astmaoireisten lasten pienten hengitysteiden toiminnan arviointiin sekä pieniin hengitysteihin suunnattujen hoitointerventioiden seurantaan.
1 INTRODUCTION

Asthma is one of the most common chronic diseases and presents a globally significant disease burden by affecting approximately 358 million people worldwide. The World Health Organization Global Burden of Disease Study 2015 estimated that asthma accounted for a loss of up to 26.2 million (95% confidence interval (CI) 20.5-32.6 million) disability-adjusted life-years and 397 000 (95% CI 363 000-439 000) deaths annually. Increasing evidence indicates that rather than being a single disease, asthma is a syndrome that encompasses a heterogeneous spectrum of different disease presentations. Asthma can develop at any age, but in approximately half of the patients, first symptoms appear during early childhood. However, different obstructive respiratory symptoms, such as wheezing and persistent cough, are extremely common during early childhood, and identifying children who go on to develop asthma is challenging.

Increasing interest has focused on the peripheral lung zone alongside progress of commercially available devices for assessing the small airways and development of small-particle asthma control medications with the potential of reaching the lung periphery. As a result, small airways have been shown to be the major site of disease pathology in different respiratory diseases such as cystic fibrosis, chronic obstructive pulmonary disease, and asthma. Various physiological measures postulated to reflect small airway properties exist, but standardization in their clinical use is lacking. Therefore, these measures are mainly restricted to research and specialized centres, and the diagnosis and monitoring of asthma focus on measures that mainly reflect large airway properties.

This thesis investigates different indices proposed to reflect small airway properties with regard to their relation to respiratory symptoms suggestive of asthma, symptom control, and later lung function, as well as their utility in clinical practice. The main focus is on impulse oscillometry indices related to small airway function, for which we define reference equations for their baseline values as well as the cut-off limits for significant bronchodilator responses and between-visit variability.
2 REVIEW OF THE LITERATURE

2.1 Development and anatomy of the respiratory system

Development of the respiratory system begins during the embryonic stage (4-7 gestational weeks) as the primitive lung bud emerges from the ventral foregut and divides into two lung buds which start branching successively (Figure 1).³ The pattern of airway branching is complete at approximately the 16th gestational week, but airway length and diameter continues to increase until early adulthood.⁴ At 23-25 gestational weeks, the lungs are in a late canalicular stage characterized by the formation of a primitive blood-gas interface, capillary proliferation, extracellular remodelling, and the beginning of surfactant production.³ The saccular stage (24-36 gestational weeks) comprises the development of primitive terminal airspaces alongside the continuation of pulmonary vasculature formation. Alveolarization, where alveoli develop from the primitive alveolar sacs and ducts, begins at 36 gestational weeks and may continue up to adolescence.⁵

![Developmental stages of the respiratory system](image)

**Figure 1.** Developmental stages of the respiratory system. Modified from Baker et al.³

The lower respiratory tract begins from the trachea which divides into two systems of asymmetrically branching airways that conduct air into the lungs (Figure 2). The length and diameter of the airways decreases towards the periphery of the tracheobronchial tree. Small airways have traditionally been defined as peripheral airways with an internal diameter of less than 2 mm, which includes airways from approximately the 8th generation onwards in an adult
lung. These airways can be divided into small conducting airways (generations 8-16) and gas-exchanging acinar airways (generations 17-23). There are important structural and physiological differences between small and large airways. First, small airways lack cartilage that supports larger airways but are lined by surfactant which reduces surface tension and prevents them from collapsing at low lung volumes. Second, airway epithelium becomes thinner and mucous cells become fewer towards the lung periphery. Third, although airway diameter decreases towards the lung periphery, the exponential increase in airway number rapidly expands their collective cross-sectional area.

In children, the concept of small airways is, however, more complicated due to lung growth. Because of the age-dependency of airway diameter, a cut-off value of 2 mm represents very different airway generations depending on the age of the subject. In addition, the development of the peripheral lung region continues for several years after birth. No definition of small airways in children has been established, and most paediatric studies refer to the cut-off value of 2 mm.

Figure 2. Schematic illustration of the tracheobronchial tree. Modified from McNulty et al.
2.2 Obstructive respiratory symptoms in childhood

2.2.1 Symptoms suggestive of asthma

Airway obstruction can manifest as variable non-specific symptoms, including wheezing, shortness of breath, chest tightness, and cough. Different obstructive respiratory symptoms during early childhood are common, but only a fraction of children with respiratory symptoms will continue to have symptoms later in life and be given the diagnosis of asthma. None of the available tests can diagnose asthma in children decisively. The term wheezing disorder is commonly used to represent a heterogeneous group of young children with different clinical presentations.

2.2.1.1 Pathophysiology

Asthma is characterized by variable airway obstruction resulting from airway hyperresponsiveness (AHR) to different stimuli such as allergens, pollutants, pathogens, or exercise (Figure 3). In asthma, airway obstruction is at least partly reversible with beta2-agonist medications that act on beta2-adrenoceptors of the airway smooth muscle and induce relaxation.\(^\text{10}\) This reaction is called bronchodilator responsiveness (BDR). Short-acting beta2-receptor agonists (SABA), which are used as asthma-attack medications, bind directly at the active site of the transmembrane beta2-adrenergic receptor, generating a rapid but relatively short-lasting bronchodilation effect. In the case of salbutamol, the maximum bronchodilation is obtained within approximately 15 minutes of inhalation.\(^\text{11}\)

Another typical feature of asthma is chronic airway inflammation, which is classically defined by a T helper type 2 (T\(_h\)2) cytokine-mediated increase in the number of eosinophils. However, sputum and bronchoscopy samples have demonstrated different inflammatory profiles in subjects with asthma,\(^\text{12-14}\) suggesting diverse endotypes within the clinically similar presentations. Most asthma control medications act to reduce this inflammation. The gold standard maintenance therapy in asthma is inhaled corticosteroids (ICS), which have broad anti-inflammatory effects by acting on multiple molecular inflammatory pathways.\(^\text{15}\) Antileukotriene medications, e.g. montelukast, can also be used to inhibit the leukotriene-mediated inflammatory pathways.\(^\text{16}\) In addition, biologic therapy with specific molecular targets such as immunoglobulin E (IgE) or interleukin 5 can be used to manage specific phenotypes of asthma.\(^\text{17}\)
A subgroup of asthmatic subjects presents with airway remodeling, including epithelial injury, goblet cell hypertrophy and hyperplasia, reticular basement membrane thickening, increased airway smooth muscle mass, and angiogenesis. These changes have been associated with severe airflow limitation, BDR, and AHR. Airway remodeling has historically been considered as a consequence of long-standing inflammation that results in dysfunctional injury and repair mechanisms. However, even preschool children with asthmatic symptoms may present with airway remodeling, suggesting that chronic inflammation might not be a prerequisite for detectable histological changes, but rather a distinct process occurring in parallel with the remodeling processes. Furthermore, airway remodeling and the natural course of lung function seem to be unaffected by anti-inflammatory medications.

The pathophysiology of asthma is based on complex interactions between genetic susceptibility and environmental exposures. Based on twin studies, it is estimated that genetic factors account for approximately 40-60% of asthma susceptibility. Genome-wide association studies have identified several susceptibility genes for asthma that have mainly been associated with epithelial barrier function and immune responses. The most well-known risk genes for childhood asthma are orosomucoid 1-like 3 (ORMDL3) and gasdermin B (GSDMB), both of which are located in chromosome 17. In mice, overexpression of these genes results in an asthma-like phenotype with increased AHR as well as airway remodeling. However, all of the known gene variants have only a modest effect on asthma susceptibility (typically a risk increase of less than 50%) and are estimated to explain less than 10% of disease heritability. Therefore, increasing interest has emerged in the epigenetic mechanisms that alter gene expression without changes in DNA sequence, such as DNA methylation or histone modification. Different epigenetic changes in the peripheral blood and the respiratory tract cells have been related to asthma, but their clinical significance remains unclear.

**Figure 3.** Characteristic pathological features of asthma. Image modified from © blueringmedia / stock.adobe.com.
Substantial differences in the prevalence of asthma and allergies have been observed between populations with similar genetic origins, but different environmental exposures. Furthermore, the prevalence of asthma and allergies increases in immigrants with increasing length of residency in countries with higher disease prevalence. Although the specific mechanisms between environmental exposures and disease development are mostly unclear, several environmental risk factors for asthma, such as exposure to tobacco smoke and early respiratory tract infections caused by rhinovirus or respiratory syncytial virus, have been identified. In addition, the protective effect of a microbiome-rich environment, especially exposure to farm animals, on asthma has been well established. Nutritional factors, such as polyunsaturated fatty acids and vitamin D, might also influence the risk of asthma development.

### 2.2.1.2 Epidemiology and morbidity

Respiratory symptoms during childhood are common and heterogeneous, rendering reliable measures of asthma prevalence during childhood difficult. The largest report on asthma prevalence in children is the International Study of Asthma and Allergies in Childhood (ISAAC), which included over 400,000 children from 56 countries in its third phase in 2002-2003. A large variation in the prevalence of self-reported asthma symptoms was observed: from 2.8% (Indonesia) to 37.6% (Costa Rica) at the age of 6-7 years, and from 3.4% (Albania) to 31.2% (Isle of Man) at 13-14 years. The symptom prevalence in 13- to 14-year-old Finnish children was 19%. Based on population-based birth cohorts, almost half of children experience at least one acute wheezing episode before 6 years of age. However, only approximately one-third of children with wheezing will continue to have persistent symptoms and a diagnosis of asthma at school age.

Obstructive symptoms during early childhood present a globally significant disease burden as the leading chronic disease causing childhood morbidity measured as school absences, emergency department visits, and hospitalizations. Paediatric hospitalizations and especially mortality due to asthma have, however, been decreasing. In Finland, four confirmed asthma deaths in children (0-19 years) were reported between 1999 and 2015, resulting in a cumulative incidence of 0.19 per million person-years.

### 2.2.1.3 Phenotypes

Several studies have sought to identify the nature of different preschool wheezing phenotypes and their associations with asthma. The ground-breaking study characterizing different preschool wheezing phenotypes was the Tucson Children’s Respiratory Study, which followed an unselected birth cohort from Tucson, Arizona. The follow-up data of 826 children were used to define four distinct wheezing phenotypes: never-wheezers (52%), transient early wheezers...
(wheezing at the age of 3 years but not at 6 years) (20%), late-onset wheezers (wheezing at the age of 6 years but not at 3 years) (15%), and persistent wheezers (wheezing at the age of both 3 and 6 years) (14%). Later studies have replicated similar time-trend patterns of preschool wheezing, some with slight variations such as the Avon Longitudinal Study of Parents and Children (ALSPAC), which extended these wheezing phenotypes by introducing prolonged early (wheezing from 6 to 54 months but not thereafter) and intermediate-onset (onset of wheezing from 18 to 42 months and continuing thereafter) wheezing phenotypes based on the data of 6265 children from an unselected UK birth cohort (Figure 4). The proportion of children who experience wheezing during their preschool years has been approximately 30-45% in these unselected birth cohorts, with 30-70% of the symptoms being transient. The Children, Allergy, Milieu, Stockholm, Epidemiology (Swedish abbreviation BAMSE) cohort used a more stringent criteria to define different phenotypes of preschool asthma, showing an expectedly lower prevalence of children fulfilling these criteria by the age of 8 years, 16%. The follow-up data of the Tucson study demonstrated that late-onset and persistent wheezing were independent risk factors for asthma at the age of 22 years. Similar associations have been observed in later studies, with persistent and late-onset wheezing phenotypes identified as risk factors for decreased lung function and asthma later in life, whereas transient early wheezers have shown more subtle associations.

The classic time-trend categorization of wheezing phenotypes can only be applied retrospectively. Therefore, in 2008, the European Respiratory Society (ERS) Task Force recommended a symptom-based classification of wheezing to either episodic (viral) or multiple-trigger wheezing. Episodic wheezing was defined as wheezing only in discrete episodes, often in association with upper respiratory tract infections, and the child being asymptomatic between these episodes. Symptoms of episodic viral wheezing usually decline over time and disappear by 6 years of age. However, some individuals may continue episodic wheezing until school age or change into a multiple-trigger wheezing phenotype. The
multiple-trigger wheezing phenotype was defined as wheezing in response to different triggers, such as allergen exposure or exercise, also between the discrete exacerbation periods. This phenotype has been associated with decreased lung function, eosinophilic airway inflammation, airway remodeling, and increased risk of asthma.\textsuperscript{22,23,62} However, significant changes in the symptom patterns over time and the poor predictive values of the different wheezing phenotypes\textsuperscript{63} led to an updated recommendation of the ERS Task Force, which highlights the importance of symptom frequency and severity in disease management.\textsuperscript{64} A recent report further analysed the stability of these phenotypes based on two independent birth cohorts and observed that 60-70\% of episodic and 40-45\% of multiple-trigger wheezers outgrew their symptoms in a 2-year follow-up.\textsuperscript{65}

The Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) birth cohort followed 411 newborns of asthmatic mothers for 7 years.\textsuperscript{66} Rather than focusing on wheezing only, a wider range of troublesome lung symptoms, defined as either wheezing, whistling, breathlessness, or persistent troublesome cough, was recorded. The frequency of troublesome lung symptoms in the daily diaries of these children was associated with the genetic asthma risk variant ORMDL3.\textsuperscript{66} Furthermore, the number of predefined episodes of troublesome lung symptoms before 3 years of age was related to asthma diagnosis at the age of 7 years.\textsuperscript{67} Wheezing was not independently associated with either of these endpoints, highlighting the importance of focusing on the overall symptom burden rather than exclusively on wheezing. Similar results were obtained in the Manchester Asthma and Allergy Study (MAAS) birth cohort of 1085 children, in which troublesome cough was associated with preschool lung function and atopy independently of wheezing.\textsuperscript{68}

\section*{2.2.1.4 Clinical assessment}

Asthma is defined as obstructive respiratory symptoms together with variable expiratory airflow limitation, and typically with co-occurring chronic airway inflammation.\textsuperscript{52} In schoolchildren and adults, asthma diagnosis is based on a history of a characteristic symptom pattern, accompanied by evidence of variable airflow limitation. Obstructive respiratory symptoms during early childhood represent a heterogeneous group of different phenotypes; none of these seems stable over time or able to predict the persistence of symptoms.\textsuperscript{52,63,69} However, objective lung function testing has not been implemented in the international guidelines of asthma diagnosis in preschool children and at present, none of the available tests can definitively diagnose asthma in young children. Therefore, a probability-based approach based on multifactorial evaluation has been adopted in recent guidelines.\textsuperscript{52,64}

Several guidelines have highlighted the importance of history taking in the diagnosis of asthma in children.\textsuperscript{52,70} Asthma symptoms are non-specific, and several differential diagnoses should be taken into account.\textsuperscript{70} However, characteristic patterns of symptoms can be distinguished by their nature, timing, and response to different triggers or medications. Typical obstructive
respiratory symptoms are expiratory wheezing, shortness of breath, chest tightness, and persistent cough, which vary in time (worsening during night or early morning) and intensity (exacerbations caused by specific triggers, such as respiratory tract infection, exercise, allergen exposure, or tobacco smoke). The symptoms often display a quick response to SABAs. Furthermore, a therapeutic trial (e.g. 3 months) with ICSs can be used to support asthma diagnosis in children with persistent symptoms.

Most children and approximately 50% of adults with asthma have allergic asthma, defined by the coincidence of allergic sensitization. According to the American Thoracic Society and the European Respiratory Society, allergic asthma is characterized by symptoms that are relieved by glucocorticoids and that have a quick response to SABAs. Furthermore, a therapeutic trial (e.g. 3 months) with ICSs can be used to support asthma diagnosis in children with persistent symptoms.

Several risk scoring systems that aim to identify preschool children who will develop persistent asthma have been developed. The most widely used of these is the Asthma Predictive Index (API), which was developed based on the Tucson Children’s Respiratory Study. In the original study, the stringent API (Table 1) showed a high negative predictive value of 84.2% (95% CI 81.4-87.0%) for active asthma at 13 years, and an acceptable positive predictive value of 51.5% (95% CI 47.7-55.3%), but a low sensitivity of 14.8% (95% CI 12.1-17.5%). Similar predictive values of the API have been obtained in different patient populations. A modified API (mAPI) was developed by the Prevention of Early Asthma in Kids trial, a prospective high-risk cohort of 285 American children born to atopic mothers. In a high-risk cohort of 289 American children, the Childhood Origins of Asthma study, the mAPI showed a high positive likelihood ratio (LR+) (19, 95% CI 3.6-100) for predicting asthma diagnosis at 11 years, although the sensitivity (19%, 95% CI 9.3-28%) and negative likelihood ratio (LR-) (0.82, 95% CI 0.73-0.92) were relatively poor. In addition, preschool children with a positive mAPI seem to benefit from asthma control medications to decrease their symptoms and exacerbations. Thus, although the sensitivities of the available predictive scoring systems remain poor, a positive API or mAPI significantly increases the probability of asthma development and indicates a potential benefit from regular asthma control medication. Therefore, the scoring systems may be useful in guiding treatment decisions in young children.
Table 1. The stringent Asthma Predictive Index (API) \(^2\) and the modified Asthma Predictive Index (mAPI) \(^3\).

<table>
<thead>
<tr>
<th>Frequent episodes of wheezing during the first 3 years of life and one major or two minor criteria</th>
<th>Original stringent API</th>
<th>mAPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of wheezing episodes</td>
<td>≥3</td>
<td>≥4*</td>
</tr>
<tr>
<td>Major criteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental physician-diagnosed asthma</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Physician-diagnosed atopic dermatitis</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Allergic sensitization to ≥1 aeroallergen</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Minor criteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician-diagnosed allergic rhinitis</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Allergic sensitization to milk, egg, or peanuts</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Wheezing unrelated to colds</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Blood eosinophils ≥4%</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

*With ≥1 wheezing episodes confirmed by a physician.

Objective documentation of variable airflow limitation based on either spirometry or peak expiratory flow (PEF) measurements combined with bronchodilation, bronchial challenge, or anti-inflammatory treatment trial are recommended to confirm asthma diagnosis.\(^5\) However, forced expiratory flow measurements can be reliably performed starting from approximately 5-7 years of age.\(^7\) Potential tools for evaluation of lung function in preschool children include forced oscillation technique (FOT)/impulse oscillometry (IOS), interrupter resistance, specific airway resistance, and multiple breath washout (MBW) measurements.\(^8\) Furthermore, eosinophilic airway inflammation can be evaluated with fractional concentration of exhaled nitric oxide (FeNO) measurements.\(^8\) However, internationally accepted criteria for diagnostic lung function measurements in preschool children are lacking.\(^7\) The Finnish Current Care Guidelines recommend the use of IOS with applicable cut-off values to support asthma diagnosis in children aged ≥3 years.\(^8\)

2.2.2 Bronchopulmonary dysplasia

Bronchopulmonary dysplasia (BPD) was first described in 1967 as a chronic lung disease that developed in premature infants with respiratory distress syndrome who were treated with aggressive mechanical ventilation and high concentrations of oxygen supplementation.\(^8\) This condition, now considered as the “old BPD”, developed in slightly preterm (gestational age 32-37 weeks) infants with diffuse structural lung damage. Improvements in perinatal care, most notably surfactant replacement therapy, antenatal corticosteroids, and changes in respiratory care practices, have substantially reduced the number of the old form of BPD.\(^8\) However, advances in neonatal intensive care have also resulted in dramatic improvements in the survival of very preterm infants of even 22-24 gestational weeks, leading to a new form of BPD that
results from early arrest of lung development.\textsuperscript{88} In a very premature infant, the normal development of both alveoli and pulmonary vessels is disrupted. As a consequence, infants with BPD show a reduced number of large alveoli and dysmorphic pulmonary vasculature, leading to a decreased surface area for respiratory gas exchange.\textsuperscript{99}

Definition of the new form of BPD is based on the need for supplemental oxygen at ≥28 days after birth, encompassing variable clinical conditions with different underlying pathologies.\textsuperscript{88} It mainly affects children born at <30 gestational weeks weighing <1500 grams.\textsuperscript{88} Approximately 40\% of infants born at ≤28 gestational weeks suffer from BPD, and the incidence sharply increases with decreasing gestational age.\textsuperscript{90} Several different pre- and postnatal exposures as well as genetic susceptibility are thought to contribute to disease development.\textsuperscript{88,91} Most importantly, hyperoxia, mechanical ventilation, and intrauterine and neonatal infections have been shown to increase the risk of BPD in premature infants.\textsuperscript{88,89,91,92} Persistent inflammation is a characteristic feature in the development of BPD, however, the specific mechanisms behind BPD are largely unknown.\textsuperscript{89,91}

Prematurely born children, especially those who develop BPD, can present with respiratory symptoms and functional abnormalities that persist to adulthood.\textsuperscript{93} The non-specific respiratory symptoms in BPD, such as recurrent wheezing and persistent cough, are often misclassified as asthma.\textsuperscript{88} Furthermore, subjects with BPD can present with obstructive spirometry measurements, increased respiratory resistance, and AHR.\textsuperscript{93,94} Substantial impairment of small airway function based on spirometry, IOS, and high-resolution computer tomography in schoolchildren with a history of BPD has also been detected.\textsuperscript{95} Despite the similarities between BPD and asthma, substantial differences in the underlying disease pathologies and clinical features exist between the two diseases. Children with BPD do not typically present with signs of eosinophilic inflammation, increased exhaled nitric oxide, or increased prevalence of atopy.\textsuperscript{96,97} In addition, airway obstruction in BPD does not usually show significant BDR.\textsuperscript{97,98} Also, subjects with BPD do not seem to benefit from ICS treatment and thus, their routine use in BPD is not recommended.\textsuperscript{99,100} Although some catch-up in their lung function may occur, survivors of BPD have an increased risk for persistent lung function impairment and a chronic obstructive pulmonary disease (COPD) -like phenotype in adult life, indicating that early disruption of lung development can have lifelong consequences.\textsuperscript{901}
2.3 Assessment of small airway function and inflammation

Small airway pathophysiology can be directly assessed only with invasive techniques, such as bronchoalveolar lavage or bronchial biopsies, which have provided valuable information for understanding different disease processes, but because of their invasiveness are not applicable for routine practice. However, different functional features, such as ventilation heterogeneity, air trapping, and premature airway closure, which have been related to small airway obstruction can be evaluated with different physiological or imaging techniques. Physiological tests that have the potential to assess small airway properties include FOT/IOS, body plethysmography, spirometry, inert gas washout measurements, and extended exhaled nitric oxide (NO) measurement. In addition, although imaging techniques do not offer sufficient resolution for direct imaging of the small airways, different imaging techniques, such as high-resolution computer tomography and hyperpolarized Helium-3 magnetic resonance imaging, can be used to evaluate functional features related to small airway obstruction, including gas trapping, mosaic lung attenuation, and regional ventilation defects. However, no gold standard measurement for small airway function exists since no biomarker or functional parameter can conclusively confirm small airway involvement. Table 2 summarizes potential techniques for assessing different small airway properties.
Table 2. Summary of potential tools for assessing small airways. Modified from McNulty et al.⁷

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameters for small airways</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
</table>
| Bronchoscopy | Transbronchial biopsy or bronchoalveolar lavage | • Direct measurement of inflammation/remodeling | • Invasive  
• Few studies on the association with clinical characteristics of asthma |
| Spirometry | FEḞ_{25-75}, FEḞ_{50}, FVC/SVC | • Wide availability  
• Low cost  
• Quick and easy to perform  
• Standardized criteria | • Low sensitivity  
• Dependent on patient cooperation  
• Not specific for small airways |
| Forced oscillation technique/Impulse oscillometry | R5-20, dR/df, X5, AX, Fres | • Relatively low cost  
• Quick and easy to perform  
• Effort-independent | • Dependent on operator technique  
• Difficult to interpret |
| Body plethysmography | RV, RV/TLC, Raw loop | • Relatively wide availability  
• Good reproducibility | • Not specific for small airways  
• Dependent on patient cooperation  
• Large variability between subjects |
| Multiple breath washout | Sacin, LCI, Scond | • Effort-independent  
• Good reproducibility  
• Sacin considered specific to small airway changes | • High cost  
• Low availability  
• Relatively time-consuming  
• Variability between different equipment |
| Extended exhaled nitric oxide | CALV | • Relatively easy to perform  
• Considered specific for small airways | • Relatively time-consuming  
• Different computational models  
• Several confounding factors |
| High-resolution computed tomography | Air trapping | • Direct visualization of the functional features  
• Quick and easy to perform | • Radiation  
• High cost  
• Difficulty in interpretation  
• Lack of standardization |
| Magnetic resonance imaging | Regional ventilation defects, inhaled marker distribution | • Direct visualization of the functional features | • High cost  
• Low availability  
• Difficult to interpret  
• Lack of standardization |

Abbreviations: AX, area under the reactance curve; CALV, alveolar nitric oxide concentration; FEḞ_{25-75} and FEḞ_{50}, forced expiratory flow at 25-75% and at 50% of FVC; FEV_{1}, forced expiratory volume in 1 s; Fres, resonance frequency; LCI, lung clearance index; FVC, forced vital capacity; SVC, slow vital capacity; R5-20 and dR/df, frequency-dependence of respiratory resistance; Raw, airway resistance; RV, residual volume; Sacin and Scond, index of ventilation inhomogeneity in the acinar structures and conducting airways; TLC, total lung capacity; X5, respiratory reactance at 5 Hz.
2.3.1 Impulse oscillometry

FOT was introduced in the 1950s by DuBois et al., who applied multiple sinusoidal pressure waves of single frequencies over normal breathing. By analysing the pressure-flow relationship of the output signals, they determined the impedance of the respiratory system and its two components, respiratory resistance and reactance. A modification of the FOT, called IOS, which used pressure pulses of different frequencies, was introduced in the 1990s. Unlike spirometry, which requires forced expiration, FOT and IOS require only passive cooperation and tidal breathing of the subject. Therefore, they are especially feasible for paediatric patients, and success rates of up to 80-100% have been reported in 2- to 7-year-old children. Although guidelines for the technical performance of the FOT/IOS have been described, incomplete standardization and limited availability have slowed their wider clinical implementation.

2.3.1.1 Technical principles and parameters

IOS uses pressure pulses of different frequencies (usually 5-35 Hz) generated by an external loudspeaker that are superimposed on tidal breathing (Figure 5). Amplitude and phase differences of the output pressure and flow signals are then analysed by Fast Fourier Transformation to determine respiratory impedance, which has two components: resistance and reactance. The resistive properties of the respiratory system are the forces opposing flow, including friction of the conducting airways and the viscous properties of the chest wall and lung parenchyma. Resistance is the part of the pressure signal that is in phase with flow. Reactance consists of two opposite forces: capacitance relative to elastic forces and inertance. Elastic properties of the lung parenchyma, airways, and chest wall are forces that oppose volume distension of the system, i.e. the reciprocal of lung compliance (elastic forces = 1/compliance). The pressure swings of elastic forces follow flow swings by 90°. Inertance comprises the forces that oppose initiation and acceleration of motion, creating pressure swings that precede flow swings by 90°. Resonance frequency (Fres) is the frequency at which elastic and inertive forces are equal, and because of their opposite pressure-phase relationship, reactance is zero. At frequencies below Fres, reactance is negative and reflects the elastic properties of the respiratory tissue, whereas at higher frequencies the significance of inertial forces increases.
The major component of respiratory resistance in healthy subjects is airway resistance. Respiratory resistance at 5 Hz (R5) is considered to reflect the resistive properties of both large and small airways, whereas resistance at 20 Hz (R20) reflects only larger airways (Table 3). Frequency-dependence of respiratory resistance, which can be expressed as the difference between R5 and R20 (R5-20) or the slope between resistance and oscillation frequency (dR/df), has been suggested as a marker of the peripheral airways based on model predictions by Otis et al., which indicated that frequency-dependence of both resistance and compliance should occur in diseased lungs with heterogeneities in time constants of the parallel airways. Later mechanical modelling has recognized the effects of tissue viscoelasticity and upper airway shunt properties on the frequency-dependence of respiratory resistance, and emphasize heterogeneous rather than homogeneous constriction of the peripheral airways to result in increased frequency-dependence of resistance. These models were validated in adults and suggested that frequency-dependence of respiratory resistance is mostly sensitive to peripheral heterogeneity in frequencies <5 Hz. However, in children, who may exhibit marked frequency-dependence of respiratory resistance between 5 and 20 Hz in normal state, it is unclear how well these models apply, and the main determinants for frequency-dependence of resistance are unknown. Furthermore, the exact anatomical location corresponding to the transition between small and large airways and determining the heterogeneity reflected by the frequency-dependence of resistance remains to be elucidated. Also, besides parallel ventilation heterogeneity, other possible mechanisms that can cause frequency-dependence of resistance include upper airway wall shunting and viscoelastic properties of the respiratory tissues. Respiratory reactance values at frequencies below Fres, such as reactance at 5 Hz (X5), are considered to reflect mainly the ability of the lung periphery to restore capacitative energy. Based on mathematical modelling, elastic properties increase in the presence of heterogeneous airway narrowing, accompanied by closure of airways and air trapping in the lung periphery. Instead of assessing single data points, the integrated low-frequency (from 5 Hz to Fres) reactance area can be calculated in order to achieve a better signal-to-noise ratio. This integrated area is called the area under the reactance curve (AX).
Table 3. Main IOS parameters and their putative physiological significance.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Physiological reflection</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5</td>
<td>Resistive properties of the whole respiratory system</td>
</tr>
<tr>
<td>R20</td>
<td>Resistive properties of the large airways</td>
</tr>
<tr>
<td>R5-20, dR/df</td>
<td>Heterogeneous obstruction of the small airways</td>
</tr>
<tr>
<td>X5</td>
<td>Elastic properties of the small airways</td>
</tr>
<tr>
<td>AX</td>
<td>Integrative measure of the elastic properties of the small airways</td>
</tr>
<tr>
<td>Fres</td>
<td>Elastic properties of the small airways (equal to the inertial powers)</td>
</tr>
</tbody>
</table>

Flow resistance is inversely related to the 4th-5th power of the tube radius. Therefore, a decrease in airway diameter results in a sharp increase in respiratory resistance. In healthy adults, respiratory resistance is nearly independent of the oscillation frequency at ≥5 Hz. However, even healthy children present with frequency-dependence of resistance which decreases with growth. Obstruction of the large airways is thought to result in a uniform increase of resistance throughout 5-35 Hz, whereas small airway obstruction is postulated to cause mechanical inhomogeneities that result in negative frequency-dependence of resistance, with the most prominent increase at the lowest frequencies and little or no changes at the highest frequencies (Figure 6). In contrast to resistance, respiratory reactance is frequency-dependent in all subjects. The frequency-dependence of reactance is enhanced in obstruction, as the low-frequency reactance values become more negative. This results in increased Fres and AX. Low-frequency reactance values have been shown to be sensitive to indirect signs of small airway obstruction, such as acinar ventilation heterogeneity and air trapping. However, so far limited experimental evidence exists on the association between IOS indices and small airway obstruction, especially in young children.
Reference values for the conventional IOS indices, such as R5 and X5, have been published in paediatric populations of different ethnicities, with a relatively good agreement.106,117-123 The main changes in the oscillation mechanics during growth include a decrease in respiratory resistance as a result of an increase in airway diameter.107,109 Also the frequency-dependence of resistance seems to decrease with growth, although few studies have provided reference values for R5-20 or dR/df.106,123 Most studies in healthy children have found standing height to be the strongest independent variable for the reference equations of IOS indices, and some have found additional variables, such as age120,124 or weight,117 to improve the reference equations. Lung compliance increases (elastic properties decrease and resistance becomes less negative) with growth, resulting in lower Fres and AX. Subtle or nonexistent differences in the IOS parameters have been observed between sexes.124

2.3.1.2 Performing the measurement and assessing reliability

Consensus statements for the technical performance of FOT in preschool children have been published by the ERS and American Thoracic Society (ATS).84,107 In addition, individual studies have provided technical guidelines for the performance of IOS.125 Key features in performing IOS in children include:

1. Setup. The measurement setting (skilled personnel, video screens, etc.) should aim to make the child feel relaxed and comfortable, and the child should be familiarized with the measurement. During the measurement the child should be sitting still in a neutral posture. A nose clip and cheek support provided by the hands of the investigator are recommended to minimize pressure loss through the upper airway shunt.84

![Figure 6. Most important impulse oscillometry indices and their changes with obstruction. Abbreviations: AX, area under the reactance curve; dR/df, frequency-dependence of respiratory resistance; Fres, resonance frequency; R5 and R20, respiratory resistance at 5 and 20 Hz; R5-20, the difference between respiratory resistance at 5 and 20 Hz; X5, respiratory reactance at 5 Hz.](image-url)
2. Visual control. Data recorded for the measurement should cover a sufficient number of regular tidal breathing cycles. Most FOT/IOS manufacturers recommend using a recording of ≥20 seconds. During data acquisition, the real-time flow and impedance versus time recordings should be monitored in order to evaluate signal quality. Breathing pattern in the flow versus time tracing should be regular and quiet without signs of apnoea or hyperventilation. In addition, the impedance versus time tracing should not contain signs of artefacts, such as sudden decrease (signifying air leak) or increase (resulting from swallowing, mouthpiece obstruction, epiglottis closure, etc.), in impedance.

3. Measurement reliability. Coherence is a measure that reflects consistency between input and output signals. It is considered to reflect the validity and reliability of an IOS measurement, and can be affected by several factors such as tension, irregular breathing, coughing, air leak, swallowing, and airway obstruction. Coherence ranges from 0 to 1, with higher values indicating better measurement reliability. No generally accepted cut-off values for sufficient measurement coherence have been determined in children. Smith et al. proposed that a coherence of ≥0.6 at 5 Hz and ≥0.9 at ≥10 Hz could be used for clinical purposes as a threshold of acceptable 20-second IOS recording.

4. Reproducibility. To confirm measurement reproducibility, the mean values of ≥3 successful recordings should be used. These individual measurements should show low variability; a coefficient of variation (CV) of <10% is usually recommended for R5. In addition, accepted resistance and reactance curves should be congruent throughout the applied frequency range.

2.3.1.3 Assessing bronchial reversibility and hyperreactivity

Reversible obstruction is a hallmark of asthma, as the hyperreactive airways react to different stimuli with obstruction that is at least partly reversible with bronchodilators. IOS can be used to assess BDR and AHR in children.

The presence of reversible airway obstruction can be evaluated by measuring the changes in lung function after administration of SABA. The relief in airway obstruction manifests as a decrease in respiratory resistance and an increase in reactance (Figure 7). Due to subtle effects on bronchial tone, even healthy children may show marked responses after inhalation of a bronchodilator compared with placebo, and therefore, accurate cut-off values are important to avoid overdiagnosis. In relatively good agreement, studies in healthy children have resulted in 5th percentile cut-off values of 37-42% for the decrease in R5 in response to salbutamol. Accordingly, a cut-off value of ≥40% decrease in R5 has been proposed as an indicator of significant BDR.
AHR can be assessed by using either direct or indirect bronchial challenge tests. Direct bronchoprovocative agents, such as inhaled methacholine or histamine, bind directly on specific airway smooth muscle cell receptors and produce bronchoconstriction. The direct challenge tests are highly sensitive in detecting subjects with asthma, but their specificity remains low.\textsuperscript{127} Indirect challenge tests assess airway responses to different stimuli that induce the release of different endogenous mediators, such as leukotrienes, histamine, and prostaglandins, that generate airway smooth muscle constriction.\textsuperscript{128} These indirect stimuli include exercise, eucapnic voluntary hyperventilation, and hypertonic saline aerosols. Contrary to direct challenges, the indirect challenge tests are generally considered highly specific but less sensitive in detecting asthmatic subjects.\textsuperscript{128} A significant AHR in the indirect challenge tests is more consistently related to markers of airway inflammation than responses in the direct challenge tests.\textsuperscript{126,128} Based on studies in healthy children, an increase of ≥35-40\% in R5 in response to bronchial challenge has been suggested as an abnormal response and an indicator of significant AHR (Figure 8).\textsuperscript{84,107,129}

![Figure 7. Impulse oscillometry results of a 7-year-old girl with symptoms suggestive of asthma before and after administration of salbutamol. The curves show significant bronchodilator responsiveness expressed as a decrease of ≥40\% in respiratory resistance at 5 Hz (R5).](image-url)

![Figure 8. Impulse oscillometry results of a 6-year-old girl with symptoms suggestive of asthma before and after an exercise challenge test. The curves show significant exercise-induced bronchoconstriction expressed as an increase of ≥35\% in respiratory resistance at 5 Hz (R5).](image-url)
2.3.2 Spirometry

Due to its high reproducibility, standardization, and wide availability, spirometry is the gold standard for diagnosing and monitoring asthma from school age to adulthood.\textsuperscript{84} Reliable measurements require good cooperation and proper forced expiration technique from the subject in order to reach flow limitation. Furthermore, strict quality control is essential when evaluating measurement reliability. Successful spirometry measurements can usually be acquired in children from approximately 5-7 years of age. Acquiring acceptable and repeatable measurements in preschool children is challenging, and studies have reported wide variability of success rates, between 40\% and 90\%, among 2- to 5-year-old children.\textsuperscript{130,131}

Spirometry measures the volume and flow rate during forced expiration (Figure 9). Airway obstruction causes airflow limitation and slowing of the expiratory flow rate, which results in a disproportionate reduction of maximal flow in relation to maximal volume, and thus, the forced expiratory volume in one second (FEV\textsubscript{1}) decreases relatively more than forced vital capacity (FVC). This is seen in the concave shape of the flow-volume curve and diminished FEV\textsubscript{1}/FVC ratio in contrast to restriction, where FEV\textsubscript{1} and FVC are proportionally diminished and the FEV\textsubscript{1}/FVC ratio remains approximately normal. Thus, the presence of airway obstruction in schoolchildren and adults is evaluated based on decreased FEV\textsubscript{1}/FVC ratio, and the severity of obstruction is based on FEV\textsubscript{1}.\textsuperscript{132}
Based on theoretical models, the first part of forced expiration is considered to reflect the emptying of large airways, as the equal pressure point where pressure inside the airway is equal to the pleural pressure is located in the large airways.\textsuperscript{133} The maximal achievable flow (flow limitation) is determined by the elastic recoil pressure of the lungs and the resistance at the airways distal to the equal pressure point. With the lung volume decreasing during exhalation, flow resistance and elastic recoil pressure decrease, and the equal pressure point moves towards the peripheral airways. Thus, the second part of FVC is considered to reflect the airflow from smaller and more peripheral airways.\textsuperscript{133,134} Obstruction in the small airways causes early airway collapse and reduces the maximal expiratory flows achieved in the mid- and end-phases of the FVC, which can be evaluated with the forced expiratory flow at 50% of the FVC (\textit{FEF}$_{50}$) and the mean expiratory flow at 25-75% of the FVC (\textit{FEF}$_{25-75}$). Thus, small airway obstruction may result in diminished \textit{FEF}$_{50}$ and \textit{FEF}$_{25-75}$, even when \textit{FEV}$_1$ is hardly affected.\textsuperscript{132} However, the relatively poor repeatability and low specificity and the lack of validation for small airway function limit the clinical use of these indices.\textsuperscript{132} Furthermore, the requirement of forced exhalation and an adequate FVC for reliable interpretation limits their use in young children.

\textbf{Figure 9.} Schematic illustration of the most important flow-volume spirometry parameters. The forced expiratory flow (\textit{FEF}) indices represent the flow rate when a specific portion (25%, 50%, or 75%) of the forced vital capacity (\textit{FVC}) has been exhaled, whereas forced expiratory volume in one second (\textit{FEV}$_1$) represents the volume that has been exhaled after one second of exhalation.
2.3.3 Multiple breath washout tests

MBW tests assess ventilation distribution inhomogeneity by analysing the washout pattern of an inert marker gas. The inert marker gas can either be exogenous, such as sulphur hexafluoride, or endogenous, such as nitrogen (N₂). The measurement is performed during tidal breathing, which makes it feasible in a wide age range, including preschool children. Accordingly, relatively good success rates of approximately 70-80% have been reported in 2- to 6-year-old children, and the measurement can be performed even in infants.135 Guidelines for performing the measurements in preschool children have also recently been published.135

The total ventilation inhomogeneity can be evaluated with the number of functional residual capacity (FRC) turnovers required to reduce the inert marker gas to 1/40 (2.5%) of its starting concentration, i.e. the lung clearance index (LCI).136 LCI represents the efficiency of a marker gas washout and is considered to reflect the inhomogeneous ventilation of the small airways, the principal site of gas mixing. Thus, in the presence of inhomogeneous small airway obstruction, LCI increases. Yet, ventilation inhomogeneity anywhere in the bronchial tree may affect LCI. Ventilation inhomogeneity is affected by two different mechanisms: 1) diffusive gas mixing close to and in the acinar structures and 2) convective flow in the conducting airways. Based on mathematical models, contribution of these mechanisms can be separated by analysing the washout pattern of the marker gas over a series of breaths in phase III slope analysis (Figure 10).137 The phase III slopes for each breath are corrected for the effect of gas dilution to determine the concentration-normalized phase III slopes (SnIII), which are plotted against the lung volume turnovers (Figure 11). Two distinct parameters can be separated from the SnIII analysis: the convection-dependent ventilation inhomogeneity in the conducting airways (Scond) and the diffusion-convection-dependent ventilation inhomogeneity in the acinar structures (Sacin).136 The cut-off between the conducting and acinar airway regions in adults is estimated to be at approximately the 15th airway generation.138

At present, the methodological variation and lack of reference values for diverse populations and devices preclude the adaptation of MBW into wider clinical use. Furthermore, although MBW seems promising for the assessment of early disease changes in cystic fibrosis (CF), its clinical utility in asthma and preschool wheezing remains unclear.85,135
Figure 10. Multiple breath nitrogen washout curve, and the normalized phase III slope (SnIII) for an individual breath. Modified based on McNulty et al. 7

Figure 11. Normalized phase III slope (SnIII) analysis of a multiple breath nitrogen washout measurement. The convection-dependent ventilation inhomogeneity (Scond) is defined as the slope of SnIII from lung turnovers (TOs) 1.5 to 6. Sacin represents the contribution of diffusion-convection-interaction-dependent ventilation inhomogeneity to the SnIII (SnIII,DCDI) at first breath. Figure reproduced from Robinson et al. 136 with permission of the © European Respiratory Society 2018.
2.3.4 Extended exhaled nitric oxide measurements

Endogenous NO is formed in a reaction catalysed by three different isoforms of NO synthases.\(^{139}\) The constitutive NO synthases generate transient and short-lasting production of NO under specific physiological conditions. In contrast, airway inflammation, especially when expressed by Th2 cytokines, enhances the production of NO by inducible NO synthase in the bronchial epithelium, which results in production of high levels of NO for prolonged periods. High levels of FeNO are observed in asthma and other inflammatory lung diseases,\(^ {140}\) and the levels usually decrease during ICS treatment.\(^ {141}\) The levels of FeNO are, however, influenced by several confounding factors, such as atopy and smoking, limiting the diagnostic potential of the marker.\(^ {86}\)

Technical guidelines for the standard online method to measure FeNO at a flow rate of 50 ml/s (FeNO\(_{50}\)) have been established by the ATS/ERS joint statement.\(^ {142}\) In summary, the measurement starts with quiet breathing for approximately 5 minutes to acclimatize, after which the subject is instructed to inhale deeply, followed by an exhalation at a constant flow rate of approximately 50 ml/s. The exhalation is continued ≥6 seconds and until a NO plateau of ≥2 seconds can be identified. At least 2-3 successful measurements with low variability (≤10% for 3 measurements, and ≤5% for 2 measurements) should be performed, and their mean value calculated.

FeNO displays an inverse correlation with the exhalation flow rate.\(^ {143,144}\) Based on a theoretical two-compartment model introduced by Tsoukias and George,\(^ {145}\) the magnitude of FeNO depends on two mechanisms: alveolar NO concentration (\(C_{ALV}\)) and diffusion of NO from the bronchial wall to the exhaled air while it travels through the airways.\(^ {143}\) At high flow rates, time for gaseous diffusion from the bronchial epithelium is insufficient, and FeNO mainly reflects alveolar NO. At decreasing flow rates, contribution of bronchial NO production increases and FeNO represents the NO from the whole respiratory epithelium. Based on these principles, applications that measure FeNO at multiple exhalation flow rates have been created for assessment of alveolar and bronchial NO output separately. By plotting the NO output against the exhalation flow rate, the relationship between FeNO and exhalation flow rate can be determined, with the intercept representing bronchial NO flux (\(J_{NO}\)) and the slope representing \(C_{ALV}\) (Figure 12).\(^ {146}\) Also non-linear models have been developed (e.g. the Högman and Meriläinen Algorithm\(^ {147}\)); however, they are more challenging for the patient and have not been validated for children.\(^ {148}\)
Figure 12. Fractional exhaled nitric oxide (NO) output (exhaled NO multiplied by the exhalation flow rate) at three different flow rates (50 ml/s, 100 ml/s, and 200 ml/s) in a 9-year-old girl with symptoms suggestive of asthma. According to the two-compartment model of Tsoukias and George, the intercept of the regression line approximates bronchial NO flux ($J_{NO}$) and its slope can be used to estimate alveolar NO concentration ($C_{ALV}$).
2.4 Small airways in respiratory disease

Despite the reduced diameter of individual airways, increase in their number and collective cross-sectional area results in decreased resistance in the lung periphery. Experimental evidence of this was first described in the 1960s by Macklem and Mead, who used a retrograde catheter and detected that the resistance of the small airways (internal diameter < 2 mm) accounted for less than 10% of the total resistance below the larynx in healthy adults. However, the contribution of the small airways in the total airway resistance was observed to be substantially larger during the first years of life, producing up to 50% of airway resistance in infancy. Furthermore, it was observed that the small airways, which had little impact on respiratory resistance in healthy lungs, were in fact the major site of airflow limitation in different respiratory diseases such as COPD and CF. However, due to large reserves and lack of techniques for assessing the lung periphery, advanced disease involvement in the small airways could be present without detectable symptoms or changes in lung function measurements. Thus, the small airways were referred to as the “silent zone”. These observations led to increasing interest in developing techniques for assessment and treatment of the small airways. Thereafter, different proposed measures small airway dysfunction have been linked to clinical features of different respiratory diseases.

Transbronchial biopsies and post-mortem lung specimens have demonstrated the presence of small airway inflammation and remodeling in patients with asthma, which is supported by clinical studies showing differences in indices related to small airway function between children with asthma or recurrent wheezing and healthy controls. Shi et al. observed that IOS indices R5-20, X5, Fres, and AX as well as the spirometry index FEF25-75 significantly differed between 6- to 17-year-old children with uncontrolled asthma and healthy controls, whereas R20 and FEV1 showed no difference between the groups. Abnormalities in ventilation inhomogeneity indices, especially LCI and Scond, have also been related to asthma and multiple trigger wheezing in children. However, Sacin, which is considered to be a more specific marker of acinar ventilation inhomogeneity, seems less affected in children with asthma and wheezing. Similarly, although some studies have reported increased levels of CALV in children with asthma, others have found the parameter to be unaffected, suggesting that alveolar inflammation might be restricted to a specific subgroup of paediatric patients with asthmatic symptoms.

Small airway dysfunction has also been associated with asthma control and different clinical features of asthma, such as AHR. Rao et al. observed that decreased FEF25-75 was associated with increased asthma severity, systemic corticosteroid treatments, and asthma exacerbations in 10- to 17-year-old asthmatic children with normal FEV1. Furthermore, Simon et al. found that FEF25-75 correlated with FeNO, AHR, and BDR in asthmatic schoolchildren with normal FEV1. In the study of Shi et al., R5-20 and AX discriminated children with uncontrolled asthma from those with controlled asthma, yielding a sensitivity of 82-86% and a specificity of 84%, whereas R20 and FEV1 showed no difference between the groups.
Furthermore, the same investigators observed that R5, R5-20, and AX predicted loss of asthma control within 8-12 weeks in 7- to 17-year-old children with controlled asthma at study enrolment, with AX correctly classifying up to 91% of the children who lost asthma control during the follow-up. Similarly, Schulze et al. found that IOS indices, including X5 and R5-20, predicted asthma exacerbations within a one-year follow-up in 4- to 7-year-old children with intermittent asthma. Arikoglu et al. found that increased R5-20 predicted AHR in exercise challenge with a specificity of 90% and a sensitivity of 60% among 6- to 18-year-old children with controlled asthma. However, no association between X5 or AX and AHR was detected. Keen et al. observed that increased Scond and FeNO50 were associated with AHR in the isocapnic dry air hyperventilation challenge of 6- to 18-year-old children with allergic asthma, whereas Sacin and CALV did not differ between children with or without AHR. Furthermore, the same study found that increased LCI and Scond were related to uncontrolled asthma based on the Asthma Control Test, whereas Sacin was unrelated to asthma control. Similarly, Mahut et al. found that CALV was unrelated to asthma severity and control based on the Asthma Control Questionnaire in 10- to 17-year-old asthmatic children, whereas Puckett et al. observed that increased CALV was related to uncontrolled asthma based on the Asthma Control Test and more frequent severe exacerbation in 6- to 17-year-old asthmatic children.

CF is a progressive disorder caused by mutations in the chloride-conducting transmembrane channel, called the CF transmembrane conductance regulator, and it affects multiple organ systems. The greatest morbidity and mortality in CF is caused by its pulmonary manifestations, characterized by mucus retention, chronic infection and neutrophilic inflammation of the airways, and progressive bronchiectasis. Small airway obstruction in CF is thought to begin early in life and progresses in a large proportion of patients despite aggressive treatments. In accordance, paediatric CF patients have been shown to present with abnormalities in ventilation heterogeneity indices, including Sacin, despite normal spirometry indices.

COPD is a largely smoking-related disorder involving irreversible airflow obstruction accompanied by chronic inflammation of the airways, lung tissue, and microvasculature. Although clinical symptoms usually appear at the age of approximately 40-50 years, increasing evidence indicates that the disease may have its origins in early childhood or even before birth. In COPD, the lung periphery is the major site of disease pathology, characterized by a mixture of small airway narrowing and destruction of the lung parenchyma. Direct catheter measurements have indicated that small airway resistance in patients with COPD can be increased to up to 40-fold. In accordance, by using multidetector computed tomography McDonough et al. observed that the number of small airways (diameter 2-2.5 mm) was reduced in all severity stages of COPD, and the magnitude of the reduction increased with disease severity. Furthermore, by analysing lung specimens of patients with severe COPD who underwent lung transplantation with microcomputed tomography, they observed a reduction of 81-99.7% in the cross-sectional area and 72-89% in the number of terminal bronchioles. Also the proposed small airway IOS indices, R5-20 and AX, have been related to disease severity and exacerbations of COPD.
2.5 Tracking of lung function

The development of lung function after birth can be divided into three principal phases: 1) lung growth from birth to early adulthood, 2) plateau phase in early adulthood (at the age of approximately 20-25 years), and 3) decline of lung function. Asthmatic subjects often present with lower levels of lung function than their healthy peers and a substantial proportion of the lung function deficits are detectable in early childhood, suggesting that impairment of lung function begins early in life.

Data from birth cohort studies suggest that impairment of lung function may be detectable already in the neonatal period. The Tucson Children’s Respiratory Study followed the lung function of 123 unselected subjects and found a significant association between the maximal expiratory flow at functional residual capacity ($V_{\text{maxFRC}}$) at 2 months of age and spirometry (FEV$_1$, FEV$_1$/FVC, and FEF$_{25-75}$) from 11 to 22 years of age. The trajectories of lung function were remarkably similar in all subjects, and those in the lowest quartile for infant $V_{\text{maxFRC}}$ had persistently decreased lung function up to age 22 years. The COPSAC followed the lung function of 317 children with asthmatic mothers from the age of 1 month to 7 years, and observed that children with asthma at age 7 had lowered spirometry indices already in infancy. However, only approximately 40% of the total airflow limitation at age 7 years was present in infancy, and the remaining 60% was acquired during early childhood. The Perth cohort followed 241 unselected children and observed that although children with decreased $V_{\text{maxFRC}}$ at the age of 1 month showed reduced FEV$_1$ and FEF$_{25-75}$ at 6 years, they had reached normal lung function by 11 years, and further improvements in their lung function were seen at 18 years. However, children with asthma diagnosis at any age had slightly reduced levels of lung function throughout the follow-up, suggesting that although some individuals might show substantial recovery of lung function, the lung function deficits related to asthma might be more persistent.

By early school age, impairment of lung function is already detectable in a substantial proportion of asthmatic subjects. This impairment often persists into adulthood and some individuals show further deterioration of lung function through adolescence. Repeated spirometry measurements in 613 subjects of an unselected New Zealand birth cohort revealed persistently lowered levels of FEV$_1$/FVC from 9 to 26 years of age in those with persistent wheezing. The trajectories of FEV$_1$/FVC were similar in all groups, suggesting that the impairment of lung function had an onset before 9 years of age. Similar findings were observed by the Childhood Asthma Study, where 84 schoolchildren (age 9-12 years) with moderate to severe asthma showed irreversible decreases of FEV$_1$ and FEV$_1$/FVC in early adulthood (age 18-31 years) despite the use of asthma control medications. The Childhood Asthma Management Program (CAMP) followed 1041 American children with mild to moderate asthma from 5 to 18 years of age, and not only did these children have lower levels of FEV$_1$/FVC than their healthy peers at study enrolment, but also the differences in FEV$_1$/FVC significantly increased during the follow-up. Similar findings were reported by the BAMSE
cohort, in which early-onset preschool asthma was associated with diminished lung function (FEV₁, FEV₁/FVC, FEF₅₀, and PEF) at the age of 8 years, and all preschool asthma phenotypes (early-transient, persistent, and late-onset) showed a further decline in lung function from 8 to 16 years.

Tobacco smoking is the most important risk factor for development and progression of COPD. However, even in the absence of active smoking a subgroup of asthmatic subjects continues to develop a COPD-like clinical phenotype with irreversible airflow limitation. Up to 75% of the CAMP study subjects with mild to moderate asthma at school age showed abnormal patterns of lung function development (reduced growth and/or early decline from 5-12 years to 23-30 years), and 11% fulfilled the diagnostic spirometry criteria for COPD (post-bronchodilator FEV₁/FVC of <0.70) before 30 years of age. Reduced lung function at the study enrolment was the strongest predictor for later lung function impairment. Similar results were obtained from the MAAS study, where decreased levels of FEV₁ and FEV₁/FVC among the 346 recruited children with a history of wheezing persisted from childhood (age 7 years) to up to 50 years of age, and the trajectories of lung function were similar to those of healthy controls. Interestingly, severe asthma in childhood appeared as a stronger risk factor for fulfilling the diagnostic criteria of COPD at 50 years than tobacco smoking. Similarly, the Aberdeen What Happens Eventually to Asthmatic children Sociologically and Epidemiologically study followed an unselected birth cohort of 330 children and observed that children with asthma diagnosis at study enrolment (age 10-15 years) already showed reduced FEV₁/FVC and were more likely to develop irreversible airflow obstruction that fulfilled the diagnostic criteria of COPD at the age of 60-65 years, even when adjusted for smoking history.

In addition to childhood lung function, other well-characterized risk factors for impaired lung in adulthood include prematurity, low birth weight, obesity, and environmental exposures, especially exposure to maternal tobacco smoking during pregnancy or early childhood, active smoking, and air pollution. Although ICSs have beneficial effects on respiratory symptoms and exacerbation frequency in most asthmatic subjects, clinical trials have found them ineffective in changing the natural course of lung function or preventing the development of asthma.

In conclusion, most of the lung function impairments in asthma seem to be already present in early childhood. Part of these deficits may be congenital but a substantial proportion seems to develop during the first years of life. In a subgroup of asthmatic subjects, deterioration of lung function progresses through adolescence and may lead to irreversible obstruction similar to COPD. Although asthma control medications seem ineffective in modifying the natural course of lung function, early identification of subjects with abnormal lung growth is crucial for optimizing the follow-up and education (most importantly, avoidance of cigarette smoking) of these patients.
3 OBJECTIVES

I To create reference values for the IOS indices proposed to reflect small airway function, and to evaluate their potential for distinguishing young children with different phenotypes of lower respiratory tract morbidity from healthy controls.

II To determine cut-off values for significant BDR and between-visit changes in the IOS indices proposed to reflect small airway function in young children.

III To investigate small airway dysfunction in children with mild to moderate symptoms suggestive of asthma compared with healthy controls by using IOS, spirometry, multiple breath nitrogen washout (MBNW), and $C_{ALV}$, and to evaluate the association of small airway dysfunction with asthma control and AHR.

IV To evaluate the longitudinal association between preschool IOS and spirometry in adolescence.
4 METHODS

4.1 Study subjects and designs

Study I

The healthy sample in this study was based on a previous study which established reference values for the conventional IOS indices. The children were recruited from seven kindergartens in Espoo, Finland. Definitions of the GAP conference for healthy children were used: all children were of Caucasian origin, born at ≥36 gestational weeks, had an appropriate growth pattern, and had no past or present chronic respiratory tract diseases or other chronic diseases. In addition, a detailed questionnaire and SPTs were used to exclude children with asthma symptoms or allergic sensitization. Raw data of the IOS measurements were collected and reanalysed to determine R5-20, the relative difference between R5 and R20 (R5-20%), and AX. The baseline IOS measurements of the healthy sample were used to establish reference values for these parameters. Altogether 103 healthy children (aged 2-7 years) were included in the study.

The symptomatic children in Study I were patients (aged 3-8 years) of the Skin and Allergy Hospital with late-onset troublesome lung symptoms (TLS), a history of early wheezing (EW), or a history of BPD who had participated in a study investigating AHR in children. Children in the TLS group (n=20) had symptoms suggestive of asthma, such as recurrent wheezing, shortness of breath during exercise, or persistent troublesome cough, which had appeared after the age of 3 years. Children in the EW group (n=37) were recruited from the subjects of a previous study investigating respiratory symptoms and lung function in infants with persistent or recurrent wheezing and/or dyspnoea at the age of ≤3 years. In addition, 8 children with a history of BPD were recruited. BPD was defined as the need for supplemental oxygen for ≥28 days after birth. Raw data of the IOS measurements of symptomatic children were collected and reanalysed to determine R5-20, R5-20%, and AX. Baseline IOS measurements of symptomatic children were compared with those of healthy children.

Study II

After the baseline IOS measurements, the healthy children described in Study I were randomized in a double-blind fashion to receive either salbutamol (n=84) or placebo (n=19). Changes after receiving salbutamol were used to evaluate the responses to bronchodilation in R5-20, R5-20%, and AX, and changes after placebo were used to evaluate the between-test repeatability of IOS.
The symptomatic children described in Study I performed IOS on ≥2 different occasions. From the children with symptoms suggestive of asthma, i.e. the TLS and EW groups, those with IOS measurements performed within a 7- to 14-day interval were selected to evaluate the within-test and between-visit repeatability of IOS. The children were free from respiratory tract infections and asthma exacerbations between the two lung function measurements, and no treatment interventions were introduced. In total, 43 children met these criteria and were included in Study II.

Study III

The patient sample in Study III included 58 children (aged 5-10 years) with symptoms suggestive of asthma: recurrent (≥3 distinct episodes) wheezing or persistent (lasting continuously for ≥6 weeks) troublesome cough without indications of alternative differential diagnoses. These children were recruited from among patients of the Skin and Allergy Hospital who were referred to lung function measurements in order to confirm their asthma diagnosis. The symptomatic children performed IOS, spirometry, and exercise challenge tests at the first visit. MBNW and FeNO measurements were performed at the second visit, occurring 1-14 days later.

Nineteen healthy controls (aged 5-10 years) were recruited using the following inclusion criteria: 1) no symptoms or diagnosis of an acute or chronic disease of the respiratory system, and 2) no systemic disease with possible direct or indirect effects on the respiratory system. The healthy controls performed MBNW, FeNO, IOS, and spirometry tests, respectively, at one visit.

Parents of the study children filled in a detailed questionnaire on the child’s family history, health status, and possible respiratory or allergy symptoms. Asthma control was assessed in the questionnaire based on: 1) the Childhood Asthma Control Test (C-ACT, Table 4) which was translated into Finnish or Swedish, 2) the use of SABA medications in the past month, and 3) the number of asthma exacerbations, i.e. episodes of shortness of breath or wheezing, in the past year. The C-ACT is a validated questionnaire for 4- to 11-year-old asthmatic children and their parents, in which a score of ≤19 indicates inadequate asthma control.
### Table 4. Childhood Asthma Control Test (C-ACT).207

<table>
<thead>
<tr>
<th>Questions for the child:</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. How is your asthma today?</td>
<td>(0) Very bad</td>
</tr>
<tr>
<td></td>
<td>(1) Bad</td>
</tr>
<tr>
<td></td>
<td>(2) Good</td>
</tr>
<tr>
<td></td>
<td>(3) Very good</td>
</tr>
<tr>
<td>2. How much of a problem is your asthma when you run, exercise, or play sports?</td>
<td>(0) It’s a big problem, I can’t do what I want to do</td>
</tr>
<tr>
<td></td>
<td>(1) It’s a problem and I don’t like it</td>
</tr>
<tr>
<td></td>
<td>(2) It’s a little problem but it’s okay</td>
</tr>
<tr>
<td></td>
<td>(3) It’s not a problem</td>
</tr>
<tr>
<td>3. Do you cough because of your asthma?</td>
<td>(0) Yes, all the time</td>
</tr>
<tr>
<td></td>
<td>(1) Yes, most of the time</td>
</tr>
<tr>
<td></td>
<td>(2) Yes, some of the time</td>
</tr>
<tr>
<td></td>
<td>(3) No, never</td>
</tr>
<tr>
<td>4. Do you wake up during the night because of your asthma?</td>
<td>(0) Yes, all the time</td>
</tr>
<tr>
<td></td>
<td>(1) Yes, most of the time</td>
</tr>
<tr>
<td></td>
<td>(2) Yes, some of the time</td>
</tr>
<tr>
<td></td>
<td>(3) No, never</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Questions for the caregiver:</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. During the last 4 weeks how many days did your child have any daytime asthma symptoms?</td>
<td>(0) Every day</td>
</tr>
<tr>
<td></td>
<td>(1) 19-24 days</td>
</tr>
<tr>
<td></td>
<td>(2) 11-18 days</td>
</tr>
<tr>
<td></td>
<td>(3) 4-10 days</td>
</tr>
<tr>
<td></td>
<td>(4) 1-3 days</td>
</tr>
<tr>
<td></td>
<td>(5) Not at all</td>
</tr>
<tr>
<td>6. During the last 4 weeks how many days did your child wheeze during the day because of asthma?</td>
<td>(0) Every day</td>
</tr>
<tr>
<td></td>
<td>(1) 19-24 days</td>
</tr>
<tr>
<td></td>
<td>(2) 11-18 days</td>
</tr>
<tr>
<td></td>
<td>(3) 4-10 days</td>
</tr>
<tr>
<td></td>
<td>(4) 1-3 days</td>
</tr>
<tr>
<td></td>
<td>(5) Not at all</td>
</tr>
<tr>
<td>7. During the last 4 weeks how many days did your child wake up during the night because of asthma?</td>
<td>(0) Every day</td>
</tr>
<tr>
<td></td>
<td>(1) 19-24 days</td>
</tr>
<tr>
<td></td>
<td>(2) 11-18 days</td>
</tr>
<tr>
<td></td>
<td>(3) 4-10 days</td>
</tr>
<tr>
<td></td>
<td>(4) 1-3 days</td>
</tr>
<tr>
<td></td>
<td>(5) Not at all</td>
</tr>
</tbody>
</table>

### Study IV

The medical records of the Skin and Allergy Hospital patients with an asthma diagnosis and a spirometry measurement performed at the age of 12-18 years between 2006 and 2008 were reviewed. The asthma diagnosis of these patients had been confirmed in the follow-up by a paediatric allergist based on their respiratory symptoms and age-appropriate lung function measurements. Patients with data on an IOS measurement performed at the age of 2-7 years
were selected to evaluate the longitudinal association between the lung function measurements. In total, 154 subjects were included in Study IV.

Figure 13 presents the study subjects and designs of Studies I-IV.

Figure 13. Study subjects and designs. Abbreviations: BDR, bronchodilator responsiveness; BPD, bronchopulmonary dysplasia; C-ACT, Childhood Asthma Control Test; EW, early wheezing; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E; IOS, impulse oscillometry; MBNW, multiple breath nitrogen washout; SPT, skin prick test; TLS, troublesome lung symptoms.
4.2 Measurements of lung function and airway inflammation

All study children were free from respiratory tract infections and asthma exacerbations ≥2 weeks prior to the lung function measurements, and SABAs were discontinued ≥12 hours before the lung function measurements. In addition, possible ICSs and other asthma control medications were discontinued ≥4 weeks before the lung function measurements in Studies I-III to minimize their confounding effect on lung function.

Impulse oscillometry

IOS was performed using similar commercial hardware (IOS, Jaeger GmbH, Würzburg, Germany), software (Masterscreen IOS, Carefusion, Hoechberg, Germany), and protocol in all studies. The measurements were performed following current international recommendations. During the measurement the child was in a sitting position and breathed quietly through a mouthpiece while wearing a nose clip. To minimize pressure losses, the hands of the investigator supported the subject’s cheeks. Pulse signals of 5-35 Hz were superimposed on the tidal breathing of the subject, and using a Fast Fourier Transformation, respiratory resistance and reactance were calculated for each oscillation frequency. The measurements were accepted when the regular breathing pattern lasted ≥20 seconds without signs of artefacts, such as air leaks, swallowing, or obstruction of the mouthpiece in the impedance versus time curve. R5-20% was calculated according to the equation: \((R5 -20)/R20*100\). At least three successful recordings were required, and their mean values were used in the analyses.

Spirometry

Spirometry measurements were performed with a pneumotachograph-based flow-volume spirometer (Masterscreen Pneumo, Carefusion, Hoechberg, Germany, in Study III, and Spiromaster Medikro Ltd., Kuopio, Finland, in Study IV) according to current international recommendations. During the measurement the child breathed through a mouthpiece in a sitting position while wearing a nose clip. The volume–time and flow–volume curves were visually inspected by the investigator, and inadequate measurements were excluded on site. At least three successful measurements meeting the acceptability and reproducibility criteria of the ATS/ERS guidelines were recorded. Regarding FEV₁ and FVC, their highest values were used in the analyses, and flow indices were derived from the recording with the largest sum of FEV₁ and FVC.
Multiple breath nitrogen washout

MBNW was performed using commercial equipment (Exhalyzer D, Ecomedics, Duernten, Switzerland) according to current international guidelines. During the measurement the child was in a sitting position, wearing a nose clip, and breathed quietly through a mouthpiece. After a period of tidal breathing of room air and ensuring breathing stability, the inhaled air was switched to 100% oxygen to evaluate the washout pattern of the endogenous N₂. To detect possible inert gas leaks during the measurement, the tidal volume and N₂ concentrations were monitored by the investigator and if signs of leaks were detected, the measurement was rejected on site. The measurement was continued until the exhaled N₂ concentration was <1/40th (2.5%) of the original starting concentration. At least two successful recordings were required and their mean values were used in the analyses.

Extended exhaled nitric oxide

FeNO measurements were recorded with a chemiluminescence analyser (CLD 88, Ecomedics, Duernten, Switzerland). First, FeNO₅₀ was measured according to international recommendations. The measurement was performed in a sitting position, and after inhalation of NO-free air, the child was instructed to exhale constantly against a 200 cm H₂O/l/s resistor. A steady exhalation at a flow rate of 40-60 ml/s lasting ≥6-10 seconds was required. The measurement was regarded as successful when ≥2 technically acceptable exhalations within 5% or 3 exhalations within 10% were acquired, and the mean FeNO₅₀ of the measurements was used in the analyses. Then, FeNO was measured at three different flow rates: 30, 100, and 200 ml/s, requiring ≥2 technically successful and reproducible measurements at each flow rate. Two-compartment linear model with three different flow rates: 200, 100, and either 30 or 50 ml/s for achieving the best goodness of fit (highest coefficient of determination, r²), was used to calculate C_ALV.

Bronchodilator response

BDR was assessed by giving the study subjects a salbutamol (Ventoline, Glaxo, UK) dose of 300 micrograms via Babyhaler® spacer (Glaxo, UK) in Study II and a dose of 400 micrograms via Volumatic® spacer (Glaxo, UK) in Study IV. The lung function measurements were repeated 10-15 minutes after the inhalation, and BDR was defined as the change in the mean value of the lung function index after inhalation. In Study II, the healthy children were randomized to receive either placebo or salbutamol, and the measurements were performed double-blinded.
Exercise challenge

Exercise challenge was performed as an outdoor free running test with either IOS or spirometry. After the baseline lung function measurements, children were urged to run 6-8 minutes with the aim of maintaining a heart rate of 85-90% of the age-dependent maximum rate. Lung function was measured 1, 5, and 10 minutes after exercise, and the test was considered as positive for a significant exercise-induced bronchoconstriction (EIB) if R5 increased $\geq 35\%^{[129]}$ or FEV₁ decreased $\geq 15\%^{[210]}$ of the baseline value.

4.3 Atopy and eosinophilia

Atopy was defined as either a positive result in SPTs or serum-specific IgE to any of the tested inhaled aeroallergens: birch, timothy grass, meadow fescue, mugwort, *Cladosporium herbarum*, cat, dog, horse, cow, or *Dermatophagoides pteronyssinus*. In Study IV, also the following food allergens were tested with SPTs: milk, egg, soy, wheat, and fish. SPTs were defined as positive if the wheal diameter was $\geq 3$ mm for $\geq 1$ of the screened allergens, with histamine dihydrochloride (10 mg/ml, ALK) serving as a positive and physiologic saline as a negative control. Serum-specific IgE was defined as positive if the level was $\geq 0.35$ kU/l for $\geq 1$ of the tested allergens.

Eosinophilia was defined as a peripheral blood eosinophil count of $\geq 0.4*10^9$/l and eosinophils composing $\geq 4\%$ of leukocytes.

4.4 Statistical analyses

Sample size

Sample size in Study III was based on power calculations for AX in Study II children with TLS. A power of 80% and type I error of 0.05 were used with the assumption that the mean of AX (standard deviation (SD) of 0.87 kPa/l) in symptomatic children would differ by 0.5 kPa/l from that in healthy controls. It was estimated that 20% additional measurements would compensate for possible unsuccessful measurements. Based on these calculations, a sample of 58 symptomatic children was recruited in the study.

The sample sizes in Studies I and II were based on the number of children from the original study samples$^{[106,126]}$ with appropriate lung function measures available. Study IV was an observational study without power calculations.
Reference values

Z-scores for the conventional IOS parameters were calculated using previously published national reference values. The reference equations defined in Study I were used to calculate z-scores for R5-20, R5-20%, and AX in Studies I and II. Z-scores for the spirometry parameters in Study IV were calculated using the national reference values. Abnormal values were defined as a z-score of ≥2 SDs for R5, R20, R5-20, R5-20%, AX, or Fres, or a z-score of ≤-2 SDs for X5, respiratory reactance at 10 Hz (X10), dR/df, FVC, FEV1, FEF50, or FEV1/FVC ratio. Due to a lack of reference values that would encompass the age range and parameters of interest in Study III, the lung function data were analysed using height- and sex-adjusted regression analyses.

Statistical analyses

Normality of the continuous variables was evaluated with Kolmogorov-Smirnov test or Shapiro-Wilk test. Accordingly, either parametric or non-parametric tests were used in the analyses. Two-tailed tests with a significance level of 0.05 were used. Data were analysed with IBM SPSS Statistics Version 21 (Study IV), 22 (Studies I and II), or 23 (Study III). Correlations between continuous variables were evaluated with Pearson’s correlations test (parametric) or Spearman’s rank correlation test (non-parametric). Differences in continuous variables between two unrelated groups were evaluated with independent-samples t-test (parametric), Mann-Whitney U test (non-parametric), or logistic regression analyses that were adjusted for relevant factors. Wilcoxon signed-rank test was used to compare related samples (e.g. baseline and post-bronchodilator IOS results). Dichotomous comparisons between groups were performed using either Chi-squared test or Fisher’s exact test.

In Study I, body surface area (BSA) was calculated with the DuBois formula: 0.007184 * weight(kg)^0.424 * height(cm)^0.725 and the Mosteller formula: (weight(kg) * height(cm) / 3600)^1/2, both applied separately for the analyses. Baseline variables with a significant association with the IOS indices were chosen to the reference model by using stepwise linear regression analysis. Thereafter, linear regression models with the highest r^2 were determined for R5-20, R5-20%, and AX based on the IOS results of the healthy reference sample. Bonferroni correction was applied for comparisons between healthy children and the patient subgroups by multiplying the p-values by the number of performed tests between the groups. Sensitivity, specificity, LR+, and LR- were calculated according to the following equations:

\[
\text{Sensitivity} = \frac{\text{true positive}}{\text{true positive} + \text{false negative}}
\]

\[
\text{Specificity} = \frac{\text{true negative}}{\text{true negative} + \text{false positive}}
\]
\[ LR^+ = \frac{\text{sensitivity}}{1 - \text{specificity}} \]

\[ LR^- = \frac{1 - \text{sensitivity}}{\text{specificity}} \]

where true positive was the number of symptomatic children with an abnormal IOS result, false positive was the number of healthy children with an abnormal IOS result, true negative was the number of healthy children with a normal IOS result, and false negative was the number of symptomatic children with a normal IOS result.

In Study II, the cut-off values for significant BDR were determined as the 5th percentile of the change after inhalation of salbutamol, expressed as the absolute change, percentage of the baseline value change (absolute change/baseline value*100%), percentage of the predicted value change (absolute change/predicted value*100%), and z-score change of each parameter. Between-test repeatability was determined in the placebo group by calculating the within-subject standard deviation (SDw) and CV for the mean values of the triplicate pre- and post-placebo measurements. The within-test repeatability was determined in symptomatic children by calculating SDw, CV, and one-way random intraclass correlation coefficient (ICC) for the triplicate baseline IOS measurements at each visit. The between-visit repeatability was evaluated by calculating SDw, CV, and two-way random ICC for the mean values of the triplicate IOS measurements at each visit. An ICC of >0.60 was considered as good agreement, and an ICC of >0.80 as excellent agreement. Low variability was defined as a CV of <15%. The cut-off value for a significant between-visit change was calculated by multiplying the between-visit SDw by 1.645.

Receiver operating characteristic (ROC) analyses in Study III were adjusted for height and sex by plotting the probability of the height- and sex-adjusted logistic regression model in the ROC curves. Associations between the proposed small airway indices (R5-20, X5, AX, FEF25-75, LCI, Scond, Sacin, and C_{ALV}) were evaluated in height- and sex-adjusted linear regression analysis.

In Study IV, asthma control medication in adolescence and baseline variables with a p-value of <0.05 in the univariate analyses investigating their association with lung function in adolescence were included in the logistic regression analysis as covariates.

### 4.5 Ethics

The studies were approved by the Research Ethics Committee of Helsinki University Hospital (approval numbers: 12/98/6§ and 337/13/03/03/2008 for Studies I and II, 390/13/03/03/2015 for Study III, and 288/13/03/03/2009 for Study IV). A written informed consent was obtained from the parents of all participating children and the children contributed an oral or written consent.
5 RESULTS

Table 5 presents the baseline characteristics and Table 6 summarizes the IOS results of the study subjects from Studies I-IV.

Table 5. Baseline characteristics of the children in Studies I-IV.

<table>
<thead>
<tr>
<th></th>
<th>Studies I &amp; II</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Patients</td>
<td>Controls</td>
<td>Patients</td>
<td>Patients</td>
</tr>
<tr>
<td>N</td>
<td>103</td>
<td>65</td>
<td>43</td>
<td>19</td>
<td>58</td>
</tr>
<tr>
<td>Male sex</td>
<td>50 (49%)</td>
<td>41 (63%)</td>
<td>31 (72%)</td>
<td>8 (40%)</td>
<td>36 (62%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>5.1</td>
<td>6.0</td>
<td>6.0</td>
<td>7.4</td>
<td>7.0</td>
</tr>
<tr>
<td>(3.7; 6.1)</td>
<td>(5.8; 6.3)</td>
<td>(5.5; 6.2)</td>
<td>(6.2; 9.3)</td>
<td>(6.2; 8.2)</td>
<td>(3.5; 5.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>109</td>
<td>118</td>
<td>118</td>
<td>130</td>
<td>125</td>
</tr>
<tr>
<td>(101; 117)</td>
<td>(112; 122)</td>
<td>(113; 122)</td>
<td>(122; 139)</td>
<td>(118; 132)</td>
<td>(98; 110)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>19</td>
<td>22</td>
<td>22</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>(16; 22)</td>
<td>(20; 24)</td>
<td>(20; 25)</td>
<td>(22; 30)</td>
<td>(21; 32)</td>
<td>(14; 19)</td>
</tr>
<tr>
<td>Atopy²</td>
<td>0</td>
<td>28 (43%)</td>
<td>20 (47%)</td>
<td>9 (47%)</td>
<td>36 (62%)</td>
</tr>
<tr>
<td>Parental atopy</td>
<td>43 (42%)</td>
<td>54 (83%)</td>
<td>36 (84%)</td>
<td>15 (79%)</td>
<td>44 (76%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>12 (12%)</td>
<td>18 (28%)</td>
<td>9 (21%)</td>
<td>0</td>
<td>21 (36%)</td>
</tr>
</tbody>
</table>

Data presented as n (n%) or median (interquartile range).

1Represents the baseline characteristics at the preschool lung function measurements.

2Skin prick test wheal diameter of ≥3 mm or serum-specific IgE of ≥0.35 kU/l for birch, timothy grass, meadow fescue, mugwort, Cladosporium herbarum, cat, dog, horse, cow, or Dermatophagoides pteronyssinus.

Table 6. Baseline IOS results of the children in Studies I-IV.

<table>
<thead>
<tr>
<th></th>
<th>Studies I &amp; II</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Patients</td>
<td>Controls</td>
<td>Patients</td>
<td>Patients</td>
</tr>
<tr>
<td>R5 kPa/l/s</td>
<td>0.90</td>
<td>0.83</td>
<td>0.82</td>
<td>0.58</td>
<td>0.79</td>
</tr>
<tr>
<td>(0.76; 1.08)</td>
<td>(0.74; 0.95)</td>
<td>(0.75; 0.91)</td>
<td>(0.50; 0.69)</td>
<td>(0.70; 0.91)</td>
<td>(0.90; 1.29)</td>
</tr>
<tr>
<td>z-score, SD</td>
<td>-0.20</td>
<td>0.07</td>
<td>0.03</td>
<td>-0.98</td>
<td>0.17</td>
</tr>
<tr>
<td>(-0.71; 0.75)</td>
<td>(-0.54; 0.93)</td>
<td>(-0.40; 0.92)</td>
<td>(-1.25; -0.16)</td>
<td>(-0.28; 0.10)</td>
<td>(-0.30; 1.71)</td>
</tr>
<tr>
<td>R5-20 kPa/l/s</td>
<td>0.13</td>
<td>0.22</td>
<td>0.22</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>(0.08; 0.19)</td>
<td>(0.16; 0.28)</td>
<td>(0.18; 0.27)</td>
<td>(0.08; 0.21)</td>
<td>(0.12; 0.29)</td>
<td></td>
</tr>
<tr>
<td>z-score, SD</td>
<td>-0.19</td>
<td>1.17</td>
<td>1.17</td>
<td>1.14</td>
<td>1.38</td>
</tr>
<tr>
<td>(-0.68; 0.56)</td>
<td>(0.27; 1.86)</td>
<td>(0.41; 1.79)</td>
<td>(0.17; 1.62)</td>
<td>(0.34; 2.47)</td>
<td></td>
</tr>
<tr>
<td>R5-20% %</td>
<td>17</td>
<td>37</td>
<td>38</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>(11; 25)</td>
<td>(25; 46)</td>
<td>(27; 46)</td>
<td>(16; 48)</td>
<td>(21; 51)</td>
<td></td>
</tr>
<tr>
<td>z-score, SD</td>
<td>-0.09</td>
<td>1.93</td>
<td>1.94</td>
<td>2.04</td>
<td>1.76</td>
</tr>
<tr>
<td>(-0.62; 0.62)</td>
<td>(-0.60; 2.62)</td>
<td>(0.72; 2.55)</td>
<td>(0.30; 2.91)</td>
<td>(0.71; 3.39)</td>
<td></td>
</tr>
<tr>
<td>X5 kPa/l/s</td>
<td>-0.27</td>
<td>0.23</td>
<td>-0.23</td>
<td>-0.18</td>
<td>-0.25</td>
</tr>
<tr>
<td>(-0.35; -0.22)</td>
<td>(-0.27; -0.19)</td>
<td>(-0.26; -0.19)</td>
<td>(-0.21; -0.16)</td>
<td>(-0.31; -0.20)</td>
<td>(-0.50; -0.29)</td>
</tr>
<tr>
<td>z-score, SD</td>
<td>0.10</td>
<td>1.99</td>
<td>1.93</td>
<td>1.01</td>
<td>1.90</td>
</tr>
<tr>
<td>(0.63; 1.41)</td>
<td>(1.37; 2.43)</td>
<td>(1.47; 2.29)</td>
<td>(0.49; 1.87)</td>
<td>(1.33; 2.67)</td>
<td></td>
</tr>
<tr>
<td>AX kPa/l/s</td>
<td>0.99</td>
<td>1.99</td>
<td>1.93</td>
<td>1.01</td>
<td>1.90</td>
</tr>
<tr>
<td>(-0.68; 0.66)</td>
<td>(-0.59; 0.63)</td>
<td>(-0.48; 0.78)</td>
<td>(-0.45; 0.63)</td>
<td>(-1.40; 0.05)</td>
<td>(-2.47; 0.18)</td>
</tr>
<tr>
<td>z-score, SD</td>
<td>-0.07</td>
<td>2.24</td>
<td>2.15</td>
<td>1.93</td>
<td>2.88</td>
</tr>
<tr>
<td>(-0.66; 0.70)</td>
<td>(1.23; 2.91)</td>
<td>(1.59; 2.85)</td>
<td>(0.59; 2.43)</td>
<td>(2.18; 3.68)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as median (interquartile range).

1Data not available for R5-20, R5-20%, or AX. dR/df was -0.02 (-0.03; -0.01) kPa/l, 1.54 (-3.35; -0.53) SDs, and Fres 23.10 (19.90; 25.98) Hz, 1.62 (0.95; 2.18) SDs.

Abbreviations: AX, area under the reactance curve; dR/df, frequency-dependence of respiratory resistance; Fres, resonance frequency; IOS, impulse oscillometry; R5, respiratory resistance at 5 Hz; R5-20 and R5-20%, absolute and relative difference between respiratory resistance at 5 and 20 Hz; X5, respiratory reactance at 5 Hz.
5.1 Reference values for R5-20, R5-20%, and AX

Natural logarithmic transformation was applied to AX, standing height, and weight to normalize their distributions. Of the investigated baseline characteristics (age, standing height, weight, BSA, and sex), standing height was the strongest independent determinant for all IOS parameters (Figure 14). R5-20 and R5-20% were also associated with sex. Adding age, weight, or BSA did not improve the goodness of fit of the regression equations. The defined regression equations are illustrated in Table 7.

![Figure 14](image)

**Figure 14.** Association between standing height and A) the difference between respiratory resistance at 5 and 20 Hz (R5-20), B) the relative difference of R5-20 (R5-20%), and C) area under the reactance curve (AX) in the healthy reference sample (n=103) from Study I. The continuous lines indicate predicted values (blue for boys, red for girls, black for both sexes) and the dashes lines illustrate the cut-off values for 2 SDs (97.7%). Ln, natural logarithm.
Table 7. Regression equations for R5-20, R5-20%, and AX based on IOS results of the healthy reference sample (n=103) in Study I.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>RSD</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5-20</td>
<td>2.057</td>
<td>-0.411</td>
<td>0.040</td>
<td>0.08240</td>
<td>0.220</td>
</tr>
<tr>
<td>R5-20%</td>
<td>136.077</td>
<td>-25.570</td>
<td>5.609</td>
<td>10.15200</td>
<td>0.123</td>
</tr>
<tr>
<td>ln(AX)</td>
<td>21.504</td>
<td>-4.587</td>
<td>–</td>
<td>0.44885</td>
<td>0.460</td>
</tr>
</tbody>
</table>

Regression equation formulated as: A + B*ln(Height, cm) + C (for boys).
Abbreviations: AX, area under the reactance curve; IOS, impulse oscillometry; ln, natural logarithm; r², coefficient of determination; R5-20 and R5-20%, absolute and relative difference between respiratory resistance at 5 and 20 Hz; RSD, residual standard deviation.
5.2 Sensitivity of IOS in detecting symptomatic children

Figure 15 illustrates the lung function of Study I children with different phenotypes of respiratory morbidity relative to healthy children. The height- and sex-adjusted z-score values of R5-20, R5-20%, and AX significantly differed between all symptomatic groups and healthy children. X5 differed from healthy children’s values only in children with a history of BPD (p=0.032), whereas R5 showed no difference between healthy and symptomatic children.

Figure 15. Z-scores of impulse oscillometry parameters A) respiratory resistance at 5Hz (R5), B) the difference between respiratory resistance at 5 and 20 Hz (R5-20), C) the relative difference of R5-20 (R5-20%), D) respiratory reactance at 5 Hz (X5), and E) area under the reactance curve (AX) in healthy and symptomatic children from Study I. ***p<0.001, **p<0.05 using Mann-Whitney U test with Bonferroni correction. BPD, bronchopulmonary dysplasia; EW, early wheezing; TLS, troublesome lung symptoms.
ROC curves illustrating the discriminative properties of R5, R5-20, R5-20%, and AX between healthy and symptomatic children from Study I are presented in Figure 16. R5-20, R5-20%, and AX differed significantly between healthy and symptomatic children, whereas R5 and X5 (p=0.982) did not differ between the groups. The number of abnormal baseline IOS indices and the sensitivity, specificity, LR+, and LR- for each IOS parameter are presented in Table 8.

Figure 16. Receiver operating characteristic curves illustrating the discriminative properties of respiratory resistance at 5 Hz (R5), absolute and relative difference between respiratory resistance at 5 and 20 Hz (R5-20 and R5-20%), and area under the reactance curve (AX) between healthy (n=103) and symptomatic (n=65) children from Study I. AUC, area under the curve; CI, confidence interval.

Table 8. Number of healthy and symptomatic children with abnormal IOS results (z-score of ≤-2 SDs for X5 and ≥2 SDs for the other parameters), and the sensitivity, specificity, LR+, and LR- for the different parameters.

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n=103)</th>
<th>Symptomatic (n=65)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5</td>
<td>2 (2%)</td>
<td>3 (5%)</td>
<td>4.6</td>
<td>98.1</td>
<td>2.4</td>
<td>0.97</td>
</tr>
<tr>
<td>R5-20</td>
<td>5 (5%)</td>
<td>13 (20%)*</td>
<td>20.0</td>
<td>95.1</td>
<td>4.1</td>
<td>0.84</td>
</tr>
<tr>
<td>R5-20%</td>
<td>2 (2%)</td>
<td>31 (48%)*</td>
<td>47.7</td>
<td>98.1</td>
<td>24.5</td>
<td>0.53</td>
</tr>
<tr>
<td>X5</td>
<td>3 (3%)</td>
<td>4 (6%)</td>
<td>6.2</td>
<td>97.1</td>
<td>2.1</td>
<td>0.97</td>
</tr>
<tr>
<td>AX</td>
<td>3 (3%)</td>
<td>36 (55%)*</td>
<td>55.4</td>
<td>97.1</td>
<td>19.0</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*P<0.05 compared with healthy children using Chi-Squared or Fisher’s exact test.
Abbreviations: AX, area under the reactance curve; IOS, impulse oscillometry; LR+ and LR-, positive and negative likelihood ratio; R5, respiratory resistance at 5 Hz; R5-20 and R5-20%, the absolute and relative difference between respiratory resistance at 5 and 20 Hz; X5, respiratory reactance at 5 Hz.
5.3 Bronchodilator responses of R5-20, R5-20%, and AX in healthy children

Of the healthy children in Study II, 84 were randomized to receive salbutamol and 19 to placebo. None of the IOS parameters of interest changed significantly after placebo as a measure of absolute, percentage of baseline, percentage of predicted, or z-score change (p>0.05 in all comparisons). The between-test repeatability measures in the placebo group were: median SDw 0.02 kPa/l/s and CV 20% for R5-20, SDw 3.31% and CV 23% for R5-20%, and SDw 0.14 kPa/l and CV 12% for AX. In contrast, all parameters decreased significantly in response to salbutamol (p<0.001 in all comparisons). Table 9 presents the 5th percentile cut-off values for the BDR in R5-20, R5-20%, and AX.

Table 9. Fifth percentile cut-off values for BDR in R5-20, R5-20%, and AX in healthy children (n=84).

<table>
<thead>
<tr>
<th></th>
<th>Absolute change</th>
<th>% of baseline change</th>
<th>% of predicted change</th>
<th>Z-score change (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5-20</td>
<td>−0.23</td>
<td>−110</td>
<td>−153</td>
<td>−2.76</td>
</tr>
<tr>
<td>R5-20%</td>
<td>−22.37</td>
<td>−106</td>
<td>−139</td>
<td>−2.20</td>
</tr>
<tr>
<td>AX</td>
<td>−2.14</td>
<td>−75</td>
<td>−137</td>
<td>−3.07</td>
</tr>
</tbody>
</table>

*Expressed as kPa/l/s for R5-20, % for R5-20%, and kPa/l for AX.

Abbreviations: AX, area under the reactance curve; BDR, bronchodilator response; R5-20 and R5-20%, the absolute and relative difference between respiratory resistance at 5 and 20 Hz.

When associations of age, height, and baseline lung function with BDR were evaluated, the absolute change in AX was significantly correlated with age, height, and baseline lung function, whereas the other measures of BDR showed associations only with baseline lung function. The association with baseline lung function was strongest regarding the absolute and z-score changes (r = −0.340 - (−0.826)), and weakest regarding the percentage of baseline value changes (r = −0.243 - (−0.340)). Figure 17 illustrates the association between BDR expressed as the percentage of baseline and baseline lung function.
Figure 17. Bronchodilator responses expressed as the relative changes of A) the difference between respiratory resistance at 5 and 20 Hz (R5-20), B) the relative difference of R5-20 (R5-20%), and C) area under the reactance curve (AX) with regard to baseline lung function. Dashed lines represent the 5th percentile cut-off values.
5.4 Repeatability of IOS in symptomatic children

Table 10 presents the within-test and between-visit repeatability of IOS within 7-14 days in symptomatic children from Study II. The within-test agreement of the three consecutive IOS measurements at both visits was excellent (ICC >0.80), and all IOS parameters showed low within-test variability (CV ≤15%). The between-visit agreement of all IOS parameters remained good (ICC >0.60) within 7-14 days, and R5 and X5 showed low between-visit variability (CV 7-11%), whereas the variability of R5-20, R5-20%, and AX was slightly higher (CV 18-24%) between the visits.

Table 10. Within-test and between-visit (7-14 days apart) repeatability of IOS among symptomatic children (n=43) from Study II.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Within-test repeatability</th>
<th>Between-test repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SDw</td>
<td>CV (%)</td>
</tr>
<tr>
<td>R5 kPa/l/s</td>
<td>0.03</td>
<td>3</td>
</tr>
<tr>
<td>z-score, SD</td>
<td>0.22</td>
<td>0.97 (0.95; 0.98)</td>
</tr>
<tr>
<td>R5-20 kPa/l/s</td>
<td>0.03</td>
<td>12</td>
</tr>
<tr>
<td>z-score, SD</td>
<td>0.36</td>
<td>0.94 (0.91; 0.97)</td>
</tr>
<tr>
<td>R5-20% %</td>
<td>5.16</td>
<td>15</td>
</tr>
<tr>
<td>z-score, SD</td>
<td>0.03</td>
<td>0.97 (0.95; 0.98)</td>
</tr>
<tr>
<td>X5 kPa/l/s</td>
<td>0.03</td>
<td>13</td>
</tr>
<tr>
<td>z-score, SD</td>
<td>0.40</td>
<td>0.92 (0.86; 0.95)</td>
</tr>
<tr>
<td>AX kPa/l</td>
<td>0.22</td>
<td>12</td>
</tr>
<tr>
<td>z-score, SD</td>
<td>0.26</td>
<td>0.96 (0.93; 0.98)</td>
</tr>
</tbody>
</table>

Data expressed as median or ICC (95% interquartile range). Within-test repeatability presented for the first visit. Abbreviations: AX, area under the reactance curve; CI, confidence interval; CV, coefficient of variation; ICC, intraclass correlation coefficient; IOS, impulse oscillometry; R5 and X5 respiratory resistance and reactance at 5 Hz; R5-20 and R5-20%, absolute and relative difference between respiratory resistance at 5 and 20 Hz; SDw, within-subject standard deviation.
5.5 Small airway IOS, spirometry, MBNW, and FeNO indices

5.5.1 Differences between healthy and symptomatic children

Table 11 summarizes the baseline lung function of Study III children. Of the proposed small airway indices, AX, which was used for the power calculations, as well as FEF25-75 and X5 differed significantly between the symptomatic children and healthy controls. Furthermore, all IOS indices, FEF25-75, and CALV differed between children with recurrent wheezing and healthy controls. However, only FEF25-75 showed a difference between children with persistent cough and healthy controls. MBNW indices showed no difference between either of the symptomatic subgroups and the healthy controls. All of the observed differences remained significant after adjusting for atopic status, except for the difference in CALV (p=0.069) between children with recurrent wheezing and healthy controls. After adjusting for parental smoking, differences in FEV1 (p=0.107) and FEF25-75 (p=0.088) did not remain significant between children with persistent cough and healthy controls, whereas the other observed differences were unaffected by the adjustment.
### Table 11. Baseline lung function of Study III children.

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n=19)</th>
<th>Recurrent wheezing (n=42)</th>
<th>p*</th>
<th>Persistent cough (n=16)</th>
<th>p*</th>
<th>All symptomatic (n=58)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IOS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5 (kPa/l/s)</td>
<td>0.58 (0.50; 0.69)</td>
<td>0.79 (0.73; 0.91)</td>
<td>&lt;0.001</td>
<td>0.79 (0.59; 1.01)</td>
<td>0.016</td>
<td>0.79 (0.70; 0.91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R5-20 (kPa/l/s)</td>
<td>0.14 (0.08; 0.21)</td>
<td>0.21 (0.15; 0.29)</td>
<td>0.047</td>
<td>0.15 (0.08; 0.32)</td>
<td>0.260</td>
<td>0.21 (0.12; 0.29)</td>
<td>0.080</td>
</tr>
<tr>
<td>X5 (kPa/l/s)</td>
<td>-0.18 (-0.21; -0.16)</td>
<td>-0.25 (-0.31; -0.20)</td>
<td>0.013</td>
<td>-0.23 (-0.29; -0.19)</td>
<td>0.073</td>
<td>-0.25 (-0.31; -0.20)</td>
<td>0.013</td>
</tr>
<tr>
<td>AX (kPa/l)</td>
<td>1.01 (0.49; 1.87)</td>
<td>1.89 (1.48; 2.67)</td>
<td>0.004</td>
<td>1.95 (0.85; 2.95)</td>
<td>0.061</td>
<td>1.90 (1.33; 2.67)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Spirometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1 (l)</td>
<td>1.67 (1.43; 2.00)</td>
<td>1.54 (1.27; 1.71)</td>
<td>0.160</td>
<td>1.45 (1.20; 1.58)</td>
<td>0.031</td>
<td>1.48 (1.24; 1.70)</td>
<td>0.054</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.89 (0.85; 0.92)</td>
<td>0.85 (0.81; 0.91)</td>
<td>0.034</td>
<td>0.86 (0.81; 0.90)</td>
<td>0.216</td>
<td>0.85 (0.81; 0.91)</td>
<td>0.031</td>
</tr>
<tr>
<td>FEF25-75 (l/s)</td>
<td>2.01 (1.63; 2.55)</td>
<td>1.59 (1.39; 1.89)</td>
<td>0.024</td>
<td>1.65 (1.33; 1.91)</td>
<td>0.045</td>
<td>1.61 (1.36; 1.90)</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>FeNO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeNO50 (ppb)</td>
<td>8.50 (4.00; 13.80)</td>
<td>12.90 (7.40; 23.33)</td>
<td>0.073</td>
<td>9.30 (4.00; 13.90)</td>
<td>0.596</td>
<td>10.80 (6.95; 21.70)</td>
<td>0.112</td>
</tr>
<tr>
<td>C_{ALV} (ppb)</td>
<td>1.35 (1.16; 2.83)</td>
<td>2.36 (1.54; 4.56)</td>
<td>0.046</td>
<td>1.44 (0.64; 2.70)</td>
<td>0.745</td>
<td>1.97 (1.38; 3.45)</td>
<td>0.138</td>
</tr>
<tr>
<td><strong>MBNW</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCI</td>
<td>6.81 (6.41; 7.33)</td>
<td>6.87 (6.52; 7.47)</td>
<td>0.455</td>
<td>6.43 (6.08; 6.55)</td>
<td>0.459</td>
<td>6.68 (6.28; 7.22)</td>
<td>0.782</td>
</tr>
<tr>
<td>Scond (1/l)</td>
<td>0.022 (0.019; 0.037)</td>
<td>0.035 (0.021; 0.042)</td>
<td>0.494</td>
<td>0.026 (0.020; 0.034)</td>
<td>0.969</td>
<td>0.022 (0.021; 0.040)</td>
<td>0.561</td>
</tr>
<tr>
<td>Sacin (1/l)</td>
<td>0.095 (0.043; 0.158)</td>
<td>0.147 (0.049; 0.192)</td>
<td>0.803</td>
<td>0.114 (0.042; 0.192)</td>
<td>0.777</td>
<td>0.124 (0.042; 0.184)</td>
<td>0.736</td>
</tr>
</tbody>
</table>

Data presented as median (interquartile range).

*P-value from the height- and sex-adjusted logistic regression analysis compared with the healthy control group.

Abbreviations: AX, area under the reactance curve; C_{ALV}, alveolar nitric oxide concentration; FEF_{25-75}, forced expiratory flow at 25-75% of the forced vital capacity; FeNO, fractional exhaled nitric oxide; FeNO_{50}, FeNO at 50 ml/s; FEV_{1}, forced expiratory volume in one second; FVC, forced vital capacity; IOS, impulse oscillometry; LCI, lung clearance index; MBNW, multiple breath nitrogen washout test; ppb, parts per billion; RS, respiratory resistance at 5 Hz; RS-20, difference between respiratory resistance at 5 and 20 Hz; Sacin, ventilation inhomogeneity in the acinar structures; Scond, ventilation inhomogeneity in the conducting airways; X5, respiratory reactance at 5 Hz.

Height- and sex-adjusted ROC analyses were used to evaluate the discriminative properties of IOS, spirometry, and C_{ALV} between children with recurrent wheezing and healthy controls (Figure 18). Of the proposed small airway indices, AX provided the best discriminative power (area under the curve (AUC) of >0.80). The unadjusted AUCs were as follows: 0.705 (95% CI 0.563-0.846, p=0.011) for R5-20, 0.777 (95% CI 0.653-0.901, p=0.001) for X5, 0.791 (95% CI 0.667-0.916, p<0.001) for AX, 0.747 (95% CI 0.614-0.881, p=0.002) for FEF_{25-75}, and 0.686 (95% CI 0.536-0.836, p=0.028) for C_{ALV}.
Figure 18. Height- and sex-adjusted receiver operating characteristic curves illustrating the discriminative properties of A) impulse oscillometry parameters difference between respiratory resistance at 5 and 20 Hz (R5-20), respiratory reactance at 5 Hz (X5), and area under the reactance curve (AX), B) spirometry parameter forced expiratory flow at 25-75% of the forced vital capacity (FEF25-75), and C) alveolar nitric oxide concentration (C_{ALV}) between children with recurrent wheezing (n=42) and healthy controls (n=19). AUC, area under the curve; CI, confidence interval.
5.5.2 Association with asthma control and AHR

C-ACT was acquired from 51 symptomatic children. The median C-ACT score was 24 (range 12-27), and 11 children (22%) had a C-ACT score of ≤19, indicating inadequate symptom control. None of the lung function parameters was associated with the C-ACT score (p>0.05).

SABA use in the past month was reported by 23 symptomatic children (40%). The use of SABA was associated with increased FeNO50 (p=0.007 in univariate analysis, and p=0.017 in height- and sex-adjusted logistic regression analysis), but not with the other lung function indices from Table 11. Sixteen children (28%) who had required SABA in the past month, reported using the medication <2 times/week, and 7 children (12%) reported a use of ≥2 times/week. Increasing SABA use was positively correlated with FeNO50 (r=0.400, p=0.004).

Of the symptomatic children, 13 (22%) reported no asthma exacerbations in the past year, 17 (29%) reported 1-3 exacerbations, 20 (35%) reported 4-12 exacerbations, and 8 (14%) reported >12 exacerbations. An increasing number of exacerbations was positively correlated with Scond, FeNO50, and C_ALV (r_s=0.340, p=0.032; r_s=0.457, p<0.001; and r_s=0.337, p=0.022, respectively). Frequent (>3/year) exacerbations were associated with increased Scond, FeNO50 (p=0.003), and C_ALV (Figure 19). The associations remained significant after adjusting for height, sex, atopy, and use of asthma control medications (p<0.05 for each comparison).

![Figure 19. Association between asthma exacerbations in the past year and A) conducting airway ventilation inhomogeneity (Scond) and B) alveolar nitric oxide concentration (C_ALV). Statistical analyses were performed with Mann-Whitney U test.](image)
Exercise challenge was performed by 55 symptomatic children (95%), 20 (36%) of whom demonstrated significant EIB. IOS was used as an endpoint measure of the exercise challenge in 29 children with 12 (41%) showing significant EIB and spirometry in 26 children with 8 (31%) demonstrating significant EIB. Positive EIB was associated with increased LCI, FeNO50 (p=0.007), and C_ALV (Figure 20). All associations remained significant in the height- and sex-adjusted logistic regression analysis (p<0.05 for each comparison).

Figure 20. Association between exercise-induced bronchoconstriction and A) lung clearance index (LCI) and B) alveolar nitric oxide concentration (C_ALV). Statistical analyses were performed with Mann-Whitney U test.

### 5.5.3 Association between the small airway IOS indices and other techniques

R5-20, X5, and AX of the Study III subjects were associated only with FEF25-75 (p≤0.001 in each comparison), not with C_ALV or MBNW indices (p>0.10 in each comparison).
5.6 Association between preschool IOS and spirometry in adolescence

Table 12 presents the longitudinal association between preschool IOS and spirometry in adolescence among the Study IV subjects with asthma.

Table 12. Correlations between the z-score values of preschool IOS and spirometry in adolescence for Study IV subjects (n=154).

<table>
<thead>
<tr>
<th></th>
<th>FVC (z-score)</th>
<th>FEV₁ (z-score)</th>
<th>FEV₁/FVC (z-score)</th>
<th>FEF₅₀ (z-score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>PostBD</td>
<td>Baseline</td>
<td>PostBD</td>
</tr>
<tr>
<td>R5 (z-score)</td>
<td>r  -0.070</td>
<td>-0.072</td>
<td>-0.253</td>
<td>-0.258</td>
</tr>
<tr>
<td></td>
<td>p  0.366</td>
<td>0.378</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>R20 (z-score)</td>
<td>r  0.003</td>
<td>-0.009</td>
<td>0.012</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>p  0.975</td>
<td>0.913</td>
<td>0.886</td>
<td>0.964</td>
</tr>
<tr>
<td>dR/df (z-score)</td>
<td>r  0.079</td>
<td>0.070</td>
<td>0.246</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td>p  0.333</td>
<td>0.388</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>X₅ (z-score)</td>
<td>r  0.067</td>
<td>0.067</td>
<td>0.081</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>p  0.412</td>
<td>0.408</td>
<td>0.319</td>
<td>0.443</td>
</tr>
<tr>
<td>X₁₀ (z-score)</td>
<td>r  0.065</td>
<td>0.059</td>
<td>0.189</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td>p  0.426</td>
<td>0.464</td>
<td>0.019</td>
<td>0.052</td>
</tr>
<tr>
<td>Fres (z-score)</td>
<td>r  0.032</td>
<td>0.043</td>
<td>-0.009</td>
<td>-0.019</td>
</tr>
<tr>
<td></td>
<td>p  0.698</td>
<td>0.599</td>
<td>0.916</td>
<td>0.817</td>
</tr>
</tbody>
</table>

Data presented as correlation coefficient (upper value) and p-value (lower value). Analyses were performed using Pearson’s correlation test with R₅, R₂₀, and Fres, and Spearman’s rank correlation test with dR/df, X₅, X₁₀, and FEF₅₀, and the statistically significant (p<0.05) correlations are indicated in boldface. Abbreviations: dR/df, frequency dependence of resistance; FEF₅₀, maximum expiratory flow when 50% of the forced vital capacity has been exhaled; FEV₁, forced expiratory volume in one second; Fres, resonance frequency; FVC, forced vital capacity; IOS, impulse oscillometry; postBD, post-bronchodilator; R₅ and R₂₀, respiratory resistance at 5 Hz and 20 Hz; X₅ and X₁₀, respiratory reactance at 5 Hz and 10 Hz.

Figure 21 illustrates the association between abnormal R₅, dR/df, or Fres in the preschool IOS measurement and post-bronchodilator lung function in adolescence. Abnormal X₁₀ was observed in 71 children and was related to decreased post-bronchodilator FEV₁ (p=0.013) and FEF₅₀ (p=0.041) in adolescence. Only three children had abnormal R₂₀, and this finding was associated with decreased FEV₁ (p=0.037) in adolescence.
Figure 21. Association between abnormal (z-score of ≥2 SDs for R5 and Fres, and ≤-2 SDs for dR/df) preschool respiratory resistance at 5 Hz (R5) (A-C), frequency dependence of respiratory resistance (dR/df) (D-F), and resonance frequency (Fres) (G-I), and post-bronchodilator (postBD) spirometry in adolescence in subjects with asthma from Study IV. Analyses were performed with Mann-Whitney U test. FEF$_{50}$, forced expiratory flow at 50% of the forced vital capacity; FEV$_1$, forced expiratory volume in one second; FVC, forced vital capacity.
Table 13 illustrates the association between abnormal IOS and spirometry when adjusted for asthma control medication in adolescence. The numbers of abnormal spirometry results were as follows: 32 for baseline FEV₁, 11 for post-bronchodilator FEV₁, 38 for baseline FEV₁/FVC, 14 for post-bronchodilator FEV₁/FVC, 29 for baseline FEF₅₀, and 13 for post-bronchodilator FEF₅₀. Neither R₂₀ nor X₅ showed significant associations with lung function in adolescence in the logistic regression model.

<table>
<thead>
<tr>
<th>Abnormal</th>
<th>Abnormal FEV₁</th>
<th>Abnormal FEV₁/FVC</th>
<th>Abnormal FEF₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>PostBD</td>
<td>Baseline</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₅</td>
<td>4.3 (1.7-10.7)</td>
<td>7.2 (1.9-26.7)</td>
<td>4.0 (1.6-9.9)</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dR/df</td>
<td>5.1 (2.1-12.3)</td>
<td>16.7 (2.1-136.1)</td>
<td>2.9 (1.3-8.3)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₁₀</td>
<td>3.7 (1.6-8.7)</td>
<td>6.8 (1.4-33.5)</td>
<td>1.9 (0.9-4.0)</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>0.018</td>
<td>0.116</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fres</td>
<td>2.6 (1.2-6.0)</td>
<td>6.3 (1.6-25.3)</td>
<td>1.7 (0.8-3.8)</td>
</tr>
<tr>
<td></td>
<td>0.021</td>
<td>0.010</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abnormal</th>
<th>Abnormal FEV₁</th>
<th>Abnormal FEV₁/FVC</th>
<th>Abnormal FEF₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>PostBD</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₅</td>
<td>2.2 (0.5-9.3)</td>
<td>0.269</td>
<td>0.046</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dR/df</td>
<td>5.8 (1.4-23.9)</td>
<td>&lt;0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₁₀</td>
<td>2.5 (0.7-8.9)</td>
<td>0.146</td>
<td>0.040</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fres</td>
<td>2.0 (0.6-6.9)</td>
<td>0.279</td>
<td>0.836</td>
</tr>
</tbody>
</table>

Data presented as correlation odds ratio with the 95% confidence interval (upper value) and p-value (lower value). Abbreviations: dR/df, frequency dependence of respiratory resistance; FEF₅₀, forced expiratory flow at 50% of the forced vital capacity; FEV₁, forced expiratory volume in one second; FEF₅₀, resonance frequency; FVC, forced vital capacity; IOS, impulse oscillometry; postBD, post-bronchodilator; R₅, respiratory resistance at 5 Hz; X₁₀, respiratory reactance at 10 Hz.
This thesis investigates different indices proposed to reflect small airway function and their utility in the assessment of young children with different phenotypes of lower respiratory tract morbidity. The association of these indices with symptom control, AHR, and later lung function was also examined. In addition, reference values for IOS indices R5-20, R5-20%, and AX as well as their cut-off values for significant BDR and between-visit variability were established.

6.1 Reference values and repeatability of R5-20, R5-20%, and AX

Despite increasing interest in the role of the small airways role in asthma, a limited number of reference values for IOS indices R5-20 and AX have been established in children. Furthermore, none of these reference values have been validated for Finnish children. Therefore, Study I established reference values for these parameters in a sample of healthy 2- to 7-year-old Finnish children. Strict selection criteria were applied to the healthy reference sample, including a detailed questionnaire and SPTs to exclude children with asthmatic symptoms or allergic sensitization. In line with previous studies, standing height appeared as the best independent variable for regression equations of all IOS parameters. Adding other anthropometric characteristics to the regression equations did not increase their goodness of fit, except for sex, which was included in the reference equations of R5-20 and R5-20% as their levels appeared slightly higher in healthy boys than in healthy girls.

Study II determined cut-off values for significant BDR of R5-20, R5-20%, and AX in the same sample of healthy children, which was used to create reference equations for their baseline values. Due to subtle effects on the bronchial tone, even healthy children might show responses to bronchodilators. Accordingly, all of the investigated IOS parameters showed marked BDR in healthy children, whereas no significant changes were seen after inhalation of placebo. This suggests that the observed changes are true responses to bronchodilator and not a consequence of intrinsic measurement variability, and underlines the importance of appropriate cut-off values for BDR to avoid overdiagnosis. Furthermore, previous studies have established even slightly higher 5th percentile cut-off values for AX (81-82% of the baseline value) using FOT. There is no clear consensus on the optimal expression of BDR, and therefore, BDR was reported as absolute, as a percentage of the baseline and predicted value, and as z-score changes.

Temporal variability in lung function is characteristic for asthma. Knowledge of the extent of this intrinsic variability is required to determine a significant change in lung function over time, e.g. in response to treatment. The within-test agreement of all investigated IOS parameters in Study II was high and variability was relatively low. The observed within-test variability was slightly lower than reported in most of the previous studies. This difference might be
related to the selection of three technically acceptable and congruent tracings on site from a total of 3-8 trials performed instead of using three consecutive IOS measurements. These results demonstrate that highly reproducible IOS measurements can be obtained in 3- to 8-year-old children.

The lung function of Study II children with symptoms suggestive of asthma was measured at two visits occurring 7-14 days apart in a time period without clinical interventions or asthma exacerbations. The between-visit variability of R5 remained low, but the proposed small airway IOS indices showed a slightly higher variability. However, the agreement of all lung function indices was good, especially when expressed as z-scores. The variability observed in Study II was slightly lower than previously reported using IOS/FOT in both healthy and symptomatic children. However, differences in study settings and populations as well as possible technical differences might explain these differences. Furthermore, an excellent agreement (ICC >0.80) in R5, X5, R5-20, and AX was observed within a 3-month follow-up in adults with stable asthma on ICS treatment. Based on these findings, IOS appears feasible for monitoring lung function over time and may potentially serve as an endpoint measure for treatment interventions targeting small airways in young children. Based on the between-visit variation observed in Study II, a decrease in R5-20, R5-20%, and AX of at least 0.65, 1.08, and 0.84 z-scores, respectively, indicates a significant treatment effect.

6.2 Small airway abnormalities in different patient groups

Studies I and III investigated the presence of small airway abnormalities in children with different phenotypes of lower respiratory tract morbidity. The discriminative properties of the proposed small airway indices differed from the parameters that mainly reflect large airway function, demonstrating their added value in detecting early lung function deficits in young children.

Study I investigated the potential of R5-20, R5-20%, and AX in distinguishing 3- to 8-year-old children with TLS, EW, or BPD from healthy controls using the reference values established in the same study. R5-20, R5-20%, and AX appeared significantly higher in all symptomatic groups than in healthy controls, whereas R5 failed to discriminate between healthy and symptomatic children. Children in the TLS group had current symptoms suggestive of asthma. These symptoms had appeared after the age of 3 years and could therefore be classified as late onset. Despite the limited duration (2-24 months) of the symptoms, these children already presented with increased levels of R5-20, R5-20%, and AX relative to healthy controls, suggesting peripheral airway impairment. No differences were, however, observed in their levels of R5 or X5 compared with healthy controls.

Although the majority of children who experience wheezing during early childhood lose their symptoms by school age, these children often manifest decreased levels of lung function also
later in life. This was supported by the results of Study I, where the EW group differed from healthy controls regarding R5-20, R5-20%, and AX, despite the disappearance of symptoms in a subgroup of these children. However, no differences were observed in R5 or X5. The lung function of children with persistent symptoms did not significantly differ from those with transient symptoms in the EW group, although a non-significant trend towards lower levels of lung function in those with persistent symptoms was observed.

Children with BPD often present with asthma-like symptoms such as wheezing, persistent cough, and AHR. Although symptoms usually remit over time, lung function of children with a history of BPD remains distinctly low throughout life, and these children are at increased risk of developing irreversible airway obstruction. In line with previous reports, children with a history of BPD in Study I differed from the healthy controls with regard to all of the proposed small airway IOS indices (R5-20, R5-20%, X5, and AX). However, no significant difference was observed in R5.

Small airway dysfunction has been associated with severe and uncontrolled asthma. However, results on the significance of small airway obstruction in early and mild disease are controversial. Study III found subtle but significant differences between 5- to 10-year-olds with recurrent wheezing and healthy controls regarding IOS and spirometry indices related to small airway function, as well as CALV. Recurrent wheezing is related to increased risk of developing asthma and persistent impairment of lung function. However, little is known about the small airway function in these children. Results from Study III indicate that, in addition to the changes in the conventional lung function indices R5 and FEV1/FVC, these children may present with early disease changes related to small airway dysfunction.

Of the investigated small airway indices in Study III, FEF25-75 was the only one that differed between children with persistent troublesome cough and healthy controls. It is possible that this group presents with little or no changes in the small airways, and these go undetected with the methods used. However, due to challenges in the differential diagnostics of persistent cough, it is possible that this patient group included also children with symptoms resulting from aetiological causes other than asthma, which might blunt the discriminative power of the analyses. Furthermore, exposure to parental tobacco smoking might also present a possible confounding aetiological factor, since the differences in spirometry indices FEV1 and FEF25-75 disappeared after adjustment for parental smoking.

In conclusion, R5-20 and AX distinguished most of the subgroups of young children with lower respiratory tract morbidities from healthy controls. These findings suggest that early disease changes in the small airways might be present in children with symptoms related to asthma and BPD.
6.3 Association of small airway function with asthma control and AHR

Study III investigated the association of small airway dysfunction with asthma control and AHR. The symptomatic children had mild to moderate symptoms and only 22% of them had an uncontrolled disease based on the C-ACT. Although the IOS indices were unrelated to asthma control and EIB, increased $C_{ALV}$ and Scond were significantly associated with asthma exacerbations in the past year, supporting that peripheral airway impairment might in some cases be related to disease control. $^{159,166,172}$ Furthermore, in accordance with previous findings on the association between small airway dysfunction and AHR, $^{161,175,176}$ increased $C_{ALV}$ and LCI were associated with EIB. However, only the FeNO indices reflecting bronchial output were associated with SABA use in the past month, and none of the investigated indices was related to the C-ACT score. These findings suggest that small airway dysfunction may have subtle, but significant associations with different clinical features of asthma in children with mild to moderate symptoms.

6.4 Small airway dysfunction at preschool age and later lung function

The tracking of lung function from early childhood to adulthood has been well established in longitudinal studies using spirometry as an endpoint measure. $^{51,188-190}$ Using IOS to measure lung function in preschool children, Study IV found that dR/df, which is postulated to reflect small airway function, had the strongest associations with lung function in adolescence. This finding is supported by two later Finnish studies. Lauhonenen et al. $^{225}$ followed 61 children with a history of bronchiolitis at <6 months of age from preschool (mean age 6.3 years) to early adolescence (mean age 11.6 years) and found that IOS indices R5 and X5 predicted later lung function assessed by spirometry. In line with these findings, Lajunen et al. $^{226}$ followed preschool children (mean age 5.0 years) with symptoms suggestive of asthma to adolescence (mean age 14.2 years), and found that preschool IOS predicted lung function in adolescence, with the strongest correlations observed between indices related to small airway function (dR/df, R5-20, Fres, and FEF$_{50}$). The findings of Study IV, Laukonen, and Lajonen remained significant with the post-bronchodilator spirometry values, suggesting that the lung function deficits may be irreversible. Moreover, the association between preschool IOS and spirometry in adolescence remained significant after adjusting for asthma control medication, supporting previous literature stating that asthma control medications seem ineffective in changing the natural course of lung function over time. $^{80,189}$
6.5 Study limitations

The main limitation of this thesis is that limited experimental evidence exists on the association between the IOS indices and small airway function, especially in young children. At present, no gold standard for assessing small airway function is available, and the influence of confounding factors, such as upper airway shunting and viscoelasticity of the respiratory tissues, on the observed changes in the proposed small airway IOS indices cannot be excluded. Nevertheless, Study III showed consistent findings in the small airway indices of IOS, spirometry, and $C_{ALV}$ despite their different methodological backgrounds, which supports the presence of true differences between the small airway properties of children with recurrent wheezing and healthy controls. However, the proposed small airway IOS indices correlated only with FEF$_{25-75}$, not with $C_{ALV}$ or MBNW indices. Lack of correlation between the indices of interest suggests that the lung function measures differ in their mechanisms or anatomical determinants relating small airway function. For example, it would have been unexpected to find correlations between measures of airway mechanics or ventilatory function determined by pressure gradients within airways, and indices related to asymmetry or inflammation of acinar lung regions where gas flow is maintained by diffusion determined by concentration gradients. Therefore, the presence of small airway dysfunction is based on theoretical assumptions and does not provide conclusive evidence of peripheral airway involvement.

Furthermore, Studies I and III included children with heterogeneous symptoms suggestive of asthma. As a substantial proportion of children grow out from their respiratory symptoms over time, these studies do not verify whether children who presented with small airway abnormalities will continue to develop asthma. However, these children represent a typical sample of patients referred to clinical assessment because of probable asthma and who could be labelled as physician-diagnosed asthma in many general practitioner-driven communities.$^{126}$ Furthermore, based on the results from Study IV and later studies with similar settings, preschool children who present with small airway abnormalities may be at an increased risk for developing impaired lung function in adolescence. Therefore, identification of these children might be important for optimizing their treatment and follow-up.

The healthy controls in Studies I and III were not perfectly matched with the symptomatic children. Therefore, height- and sex-adjusted z-scores were used to compare the healthy controls and patient groups in Study I, and height- and sex-adjusted multivariate analyses were used for the group-wise comparisons in Study III. Although the sample of healthy controls in Study III was relatively small, it resulted in a sufficient power for detecting significant differences in the postulated small airway IOS and spirometry indices as well as in $C_{ALV}$ between children with recurrent wheezing and healthy controls. These findings are in line with the previous literature, which has found small airway abnormalities to be related to different clinical features of asthma. However, as the power calculations were based on AX, it is possible that differences in other indices, such as the MBNW indices, might have been observed with larger samples.
Study II did not include data on BDR in symptomatic children. Therefore, further studies with different patient samples are required to determine the optimal measure of BDR for discriminating children with asthma from healthy controls. Repeated IOS measurements in Study II were available for symptomatic children only. Limited data on the between-test repeatability of IOS have been published in healthy children. A relatively high variability of IOS within 15 days was observed in healthy Mexican children and adolescents: CV of 10.5% for R5, 30.2% for R5-20, 26.9% for X5, and 16.7% for AX. However, IOS indices show good repeatability over months in both healthy and asthmatic adults.

The preschool lung function in Study IV was determined retrospectively in adolescents with asthma diagnosis. Therefore, the causality of the association cannot be concluded. Furthermore, the adolescents were recruited from patients followed in a tertiary hospital, which might have resulted in a selection bias towards patients with more severe disease. Accordingly, up to 21% of the patients had abnormal FEV\textsubscript{1} at baseline despite treatment. However, later prospective studies have reported similar associations, suggesting that small airway function in early childhood might predict later lung function. Asthma control in Study III was based on a questionnaire of past events. Therefore, the possibility of recall bias cannot be excluded, especially with regard to asthma exacerbations in the past 12 months.

### 6.6 Clinical implications and future considerations

This thesis established reference values for IOS parameters R5-20, R5-20%, and AX in 2- to 7-year-old Finnish children, which appeared useful in identifying children with different phenotypes of lower respiratory tract morbidity. Therefore, the reference values might be applicable for assessing small airway abnormalities in children. Furthermore, children with mild to moderate recurrent wheezing differed from healthy controls with regard to different measures related to small airway function, implying that small airway dysfunction might be present even in mild asthma in this age group. Prospective studies are needed to clarify whether subjects who present with small airway abnormalities during early childhood develop asthma. In addition, further studies should elucidate whether the children who present with abnormalities in the proposed small airway indices benefit from treatment with therapies targeting the peripheral lung region, and determine which of the available measures are optimal for identifying and following these patients.

This thesis also determined cut-off values for a significant BDR in R5-20, R5-20%, and AX. All three parameters showed marked BDR and therefore, studies with relevant patient populations are needed to determine whether these indices can be used to assess reversible obstruction in the small airways, and which measure of BDR is optimal for clinical use.

Despite the associations between small airway dysfunction and different clinical features of asthma, a limited number of studies evaluating the effect of asthma control medication targeting
the small airways has been published in children. This thesis demonstrated that agreement of the proposed small airway IOS indices remained good within 7-14 days in young children, although the variability of these indices was higher than that observed with R5. Therefore, the established cut-off values could be used as endpoint measures for studies with treatment interventions targeting the small airways in young children with symptoms suggestive of asthma.

Finally, this thesis demonstrated an association between preschool IOS and spirometry in adolescence. Interestingly, this association was strongest regarding dR/df, which is postulated to reflect heterogeneous obstruction of the small airways, and remained significant with the post-bronchodilator spirometry measurements. The small airways are the major site of obstruction in COPD and therefore, further studies are needed to clarify whether subjects who present with small airway abnormalities in early childhood are at increased risk for developing a COPD-like phenotype in adulthood.
7 CONCLUSIONS

1. Reference values for proposed small airway IOS indices were established in 2- to 7-year-old Finnish children. These indices were superior to conventional IOS indices in distinguishing children with different phenotypes of lower respiratory tract morbidity from healthy controls.

2. Cut-off values were determined for significant BDR in the proposed small airway IOS indices. In addition, within-test and between visit repeatability of IOS was investigated and cut-off values for significant between-visit changes were established. The proposed small airway IOS indices showed greater variability during follow-up than conventional IOS parameters, but their between-visit agreement remained good.

3. The proposed small airway IOS, spirometry, and FeNO indices differed between children with recurrent wheezing and healthy controls. Among symptomatic children, \( C_{ALV} \) and \( S_{cond} \) were related to frequent asthma exacerbations in the past year. Furthermore, \( C_{ALV} \) and LCI were associated with EIB. The lack of associations between most of the postulated small airway indices suggests that the mechanisms behind their relation to small airway function may be different.

4. In a longitudinal study of subjects with asthma, preschool IOS indices, especially \( dR/df \) which has been related to small airway obstruction, were associated with pre- and post-bronchodilator lung function in adolescence. The associations remained significant after adjusting for possible confounding baseline factors such as asthma control medication.
ACKNOWLEDGEMENTS

This research was carried out in 2013-2018 at the Skin and Allergy Hospital, University of Helsinki and Helsinki University Hospital, Finland. It is part of the Doctoral Programme in Clinical Research of the University of Helsinki. The research was made possible by all of the children and their families who participated in the studies.

My deepest gratitude is owed to my outstanding supervisors, Adjunct Professor Pekka Malmberg and Adjunct Professor Anne Kotaniemi-Syrjänen whose expertise, guidance, and commitment made this thesis and my growth as a researcher possible. Pekka’s support throughout my research has been invaluable and his tireless mentoring transformed the complex world of respiratory physiology to a most fascinating field of study. Anne’s tutoring started on a Sunday morning, when I was a second-year medical student, and continued as guidance in anything from statistics and scientific writing to clinical paediatrics, providing a solid basis in research and much more. I could never have imagined better mentors.

I also owe sincere gratitude to Professor Mika Mäkelä for providing me with excellent research facilities and for his valuable expertise and guidance throughout my academic career. My deepest gratitude also goes to Adjunct Professor Anna Pelkonen for her encouragement and excellent advices on my research projects. My co-authors Satu Kalliola, PhD, and Jonas Bondestam, PhD, are thanked for their collaboration and support in research and also as my clinical seniors. My sincere gratitude also belongs to Professor Seppo Sarna for guidance with statistical analyses.

I extend my sincerest gratitude to the staff of the Department of Clinical Physiology of the Skin and Allergy Hospital for performing the lung function measurements related to this thesis with the greatest skill and commitment. Special praise is due to Research Nurse Anssi Koivuselkä, whose exceptional ability to work with children and dedication to this project have been invaluable.

The official reviewers of this thesis, Adjunct Professor Heikki Lukkarinen and Adjunct Professor Kirsi Timonen are thanked for providing valuable comments to improve this thesis and for preparing me for the public examination. I want to acknowledge Carol Ann Pelli, HonBSc, for the language revision of this thesis. I also thank Adjunct Professor Päivi Piirilä and Adjunct Professor Turkka Kirjavainen for being members of my thesis committee.

I am deeply grateful to Adjunct Professor Per Gustafsson for agreeing to act as my opponent and for inspiring me with his research. I look forward to discussing this thesis with him.

My colleagues at the Pediatric Unit of the Skin and Allergy Hospital, especially Riikka Uotila, PhD; Anette Määttä, MD; Katariina Lajunen, MD; Tiina Kauppila, MD; and Kati Palosuo, PhD,
are warmly thanked for sharing many memorable moments inside and outside of the hospital and for friendship, support, and endless coffee breaks filled with laughter.

Financial support from the Research Foundation of Pulmonary Diseases, the Ida Montin Foundation, the Finnish Allergy Research Foundation, the Finnish Medical Foundation, the Foundation for Pediatric Research, the Emil Aaltonen Foundation, the Väinö and Laina Kivi Foundation, the Allergy and Asthma Federation, The University of Helsinki Research Funds, and the Sigrid Jusélius Foundation is gratefully acknowledged.

Lastly, I thank my incredible friends and family for invaluable friendship, patience, and tremendous support received during this project and beyond it. I particularly thank my brother Sami, for growing up alongside me and for teaching me persistence and determination. My grandparents, Eila, Onni, Helmi, and Ossi, for giving me fresh perspectives in life and for invaluable care and guidance throughout my life. Ali, for his love and understanding, for encouraging me in times of difficulty, for inspiring me with his astounding determination, and for sharing happiness, dreams, and passions. Finally, my parents, Taina and Olli, for their constant love and dedication, for their patience and understanding always, for believing in me, and for encouraging me to reach for my dreams.

Helsinki, October 2018

Hanna Knihtilä
REFERENCES


142. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement


174. van der Wiel E, Postma DS, van der Molen T, Schiphol-Godart L, Hacken ten NHT, van den Berge M. Effects of small airway dysfunction on the clinical expression of asthma: a focus on asthma symptoms and bronchial hyper-responsiveness. Allergy 2014;69(12):1681–8.


