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LUNG FUNCTION IN RELATION TO PRETERM BIRTH AND ASTHMA IN EARLY CHILDHOOD

Per Thunqvist

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LUNG FUNCTION IN RELATION TO PRETERM BIRTH AND ASTHMA IN EARLY CHILDHOOD
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Per Thunqvist

Principal Supervisor:
Erik Melén
Karolinska Institutet
Institute of Environmental Medicine

Co-supervisor(s):
Jenny Hallberg
Karolinska Institutet
Institute of Environmental Medicine

Mikael Norman
Karolinska Institutet
Department of Clinical Science, Intervention and Technology

Per M Gustafsson
University of Gothenburg
The Sahlgrenska Academy

Opponent:
Bill Hesselmar
University of Gothenburg
The Sahlgrenska Academy

Examination Board:
Kjell Alving
Uppsala University
Department of Women's and Children's Health

Hans Hedenström
Uppsala University
Department of Medical Sciences, Clinical Physiology

Baldvin Jonsson
Karolinska Institutet
Department of Women's and Children's Health

A famous British professor, Andrew Bush, often emphasizes – Why? How? So what? – in connection to research. The questions could be applied in wider perspectives and were central to me when I decided to become a PhD-student.

Why should a middle-aged clinician, without the need for an additional career, double the workload?
During the years as a clinician, I have often been concerned about the lack of knowledge on how to care for and how to treat the respiratory symptoms seen in growing children born preterm. In the evolving research friendly environment at Sachsska, I realized that I had the possibility to in some way contribute to that "black whole".

How?
The "perfect storm" struck me. The hospital provided the infant lung function equipment. My colleges Magnus and Gunnar got me started. Jenny and Erik were the perfect catalyst.

So what?
Personally, this has been a joyful experience and given me new insights, not only to research. Hopefully, some of the results of the studies in the thesis actually put some new knowledge into the "black whole" I wanted to explore.

Per Thunqvist 2016

ABSTRACT

The prevalence of preterm birth (ie., before 37 weeks of gestation) is increasing and estimated to be around 11 % worldwide. In high-income countries, survival is the most probable outcome even after extreme preterm deliveries. Children born preterm exhibit different stages of lung immaturity at birth and follow-up studies have shown lung function impairment and respiratory morbidity, including asthma-like disease, during infancy and into adulthood. Asthma or asthma-like disease is common in the general pediatric population. Differentiation of early childhood asthma phenotypes demonstrates different underlying pathophysiologic mechanisms and trajectories with age.

The general aims of the present thesis were:
- To investigate lung function from infancy to adolescence in relation to preterm birth and asthma in early childhood.
- To investigate if similarities and differences in measured lung function after preterm birth and among childhood asthma phenotypes may give information on mechanisms for the obstructive patterns seen in these different conditions.

Longitudinal follow-up during the first 18 months of life of infants with bronchopulmonary dysplasia (BPD) showed significant reductions of lung function in the cohort. Maximal expiratory flows were on average below the 2.5th percentile for both mild and moderate/severe BPD at both 6 and 18 months, with the exception $V_{max}FRC$ at 6 months and no improvement was seen between the two time-points. Compliance of the respiratory system (CO_{so}) was the only lung function variable that differed statistically between mild and moderate/severe BPD, with lower values for the more severe disease. Significant lower values were consistently seen for C_{so} and all maximal expiratory variables in infants with respiratory symptoms compared to infants without.
In a follow-up of children born extremely preterm (before gestational week 27), a subset of the population based cohort EXPRESS, performed lung function at age 6½ years. The extreme preterm children had lower forced expiratory volume in 1 sec (FEV_1, z-score: -1.1, 95% CI: -1.4; -0.8) than the control group born at term. Impulse oscillometry (IOS) measurements showed significantly higher peripheral airway resistance and reactance in children born extremely preterm than in controls. In children born at 22-24 weeks of gestation, 44% had FEV_1 below the <5th percentile.

Using the BAMSE cohort study, lung function after moderate-to-late preterm birth was investigated at 8 and 16 years of age. At age 16 years, both genders in the preterm group demonstrated lower FEV_1 (female subjects: –116 mL [95 % CI: –212 to –20]; male subjects: –177 mL [95 % CI: –329 to –25]) compared with the term group. IOS demonstrated higher frequency dependence of resistance (R_{5-20}) for male subjects (20.9 Pa・L–1・s−1 [95 % CI: 9.8 to 31.9]) compared with the term group. No catch up of lung function between ages 8 and 16 years was observed in either gender.

In the last study, children taking part in the BAMSE cohort study were categorized into 'never asthma', 'early transient asthma', 'early persistent asthma', and 'late onset asthma' and lung function data from the 8 and 16 year follow-ups was used to compare groups. Compared with the never asthma group, all asthma groups were associated with lower FEV_1 at 16 years of age (early transient—119 ml, 95% confidence interval 204 to 34; early persistent—410 ml, 95 %CI 533; 287; and late onset—148 ml, 95%CI 237; 58). R_{5-20} was significantly associated with active asthma at 16 years, but not transient asthma.

In conclusion:

- A significant proportion of children born very and extreme preterm have lung function values below the normal range in infancy and at at early scool-age. Lung function in adololescense is lower after moderate-to-late preterm birth than after term.
- Staging BPD severity did not predict lung function.
- Early measured lung function is associated with respiratory morbidity in children with BPD.
- IOS identifies children with low lung function after preterm birth and those with active pediatric asthma.

Clinical remark:

Measurements of lung function may identify children at risk for respiratory morbidity and provide insights into long-term sequel of preterm birth. Regular assessments of lung function from infancy, during childhood and possibly throughout life, are therefore suggested to be an important tool when monitoring individuals born preterm.

LIST OF SCIENTIFIC PAPERS

I. **Thunqvist Per**, Gustafsson Per M, Norman Mikael, Wickman Magnus, Hallberg Jenny.
Lung function at 6 and 18 months after preterm birth in relation to severity of bronchopulmonary dysplasia. Pediatr Pulmonol. 2015 Oct;50(10):978-86.

II. **Thunqvist Per**, Tufvesson Ellen, Bjermer Leif, Fellman Vineta, Domellöf Magnus, Melén Erik, Norman Mikael, Hallberg Jenny.
Lung function in 6-year-old children born extremely preterm - a population-based cohort study (EXPRESS). Submitted.

III. **Thunqvist Per**, Gustafsson Per, Schultz Erica, Bellander Tom, Berggren-Brostrom Eva, Norman Mikael, Wickman Magnus, Melén Erik, Hallberg Jenny.
Lung Function at 8 and 16 years after Moderate-to-Late Preterm birth: A Prospective Cohort Study. Pediatrics. 2016 Mar 23. pii: peds.2015-2056.

IV. Hallberg Jenny, **Thunqvist Per**, Schultz Erica S, Kull Inger, Bottai Matteo, Merritt AS, Chiesa Flaminia, Gustafsson Per M, Melén Erik.
Asthma phenotypes and lung function up to 16 years of age-the BAMSE cohort. Allergy. 2015 Jun;70(6):667-73.

CONTENTS

LIST OF ABBREVIATIONS

BPD	Bronchopulmonary Dysplasia
AX	Area of reactance
BAMSE	Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology
CPAP	Continuous Positive Airway Pressure
Cso	Single occlusion method for compliance of the respiratory system
EXPRESS	Extremely Preterm Infants in Sweden Study
fdR	Frequency dependence of resistance, R5-20
$FEV_{0.5}$	Forced expiratory volume at 0.5 seconds
FEV_1	Forced expiratory volume at 1 second
FOT	Forced Oscillation Technique
FRC	Functional Residual Capacity
FVC	Forced Expiratory Volume
IFLT	Infant Lung Function Test
IOS	Impulse Oscillometry, a variant of FOT (see below)
MEF_{50}	Maximal expiratory flow at 50 % of FVC
R_{20}	Resistance at 20 Hz
R_5	Resistance at 5 Hz
RVRTC	Raised Volume Rapid Thoracic Compression, “raised squeeze”
TVRTC	Tidal Volume Rapid Thoracic Compression, “tidal squeeze”
$V'_{max}FRC$	Maximal forced expiratory flow at FRC
X	Reactance
Zrs	Respiratory system impedance

1 INTRODUCTION

Early life exposures and insults to the respiratory system are recognized to have consequences on respiratory health during childhood and in later life.(1, 2) Preterm birth is a common event that has impact on lung function development and respiratory health during childhood and into adulthood.(3, 4) The burden of health from preterm birth, defined as birth before 37 (≤ 36+6) completed weeks of gestation, is substantial and increasing in most regions of the world that have access to reliable data.(5) The prevalence of preterm birth is estimated to be around 11 percent worldwide and ranging from 18 percent in some African countries to five percent in high-income countries like Sweden. A further division of preterm birth into extremely preterm (< 28 weeks), very preterm (28 to <32 weeks), and moderate-to-late preterm (32 to <37 weeks) is important, since lower gestational age is associated with increasing mortality and morbidity. The vast majority are moderate-to-late preterm births, while 1 – 2 percent are born very or extremely preterm.(5, 6) In the Swedish national study cohort EXPRESS (Extremely Preterm Infants in Sweden Study) the incidence of extremely preterm birth (< 27 weeks of gestation) was 3/1 000 infants.(7) Further, infant survival is nowadays the most probable outcome after extremely preterm birth (8) and the burden of respiratory morbidity after preterm birth could therefore be assumed to increase in the future. Very preterm birth (< 32 weeks of gestation) is associated with three times increased risk of childhood wheezing disorder compared with term birth.(4) Respiratory disease after preterm birth shares some clinical and pathophysiological features with asthma, such as airway obstruction, airway hyper-responsiveness and susceptibility to respiratory wheezing.(9, 10) Even though asthma is now described as a heterogeneous disease, it is usually characterized by chronic airway inflammation (GINA guidelines 2015). Airway inflammation is not a feature of the asthma-like disease seen after preterm birth and it has been suggested that the word asthma should be avoided when describing the chronic pulmonary disease seen after preterm birth.(11, 12)

Asthma or asthma-like syndrome after term birth is common in young children and prevalence of current asthma is reported to be 8 – 9 % at school age.(13) Prevalence estimations in younger children are often based on epidemiological studies that include data on respiratory wheeze.(14) To aid in management and treatment of asthma or asthma-like disease, a number of asthma phenotypes has been suggested.(15) Epidemiological studies have identified asthma phenotypes with early onset of asthma-like symptoms and impairment

of lung function that track throughout life, not only in those with persisting symptoms, but also after early transient symptoms.(16) The transient early wheeze phenotype is common during the first years of life, with declining prevalence during the preschool period, and is not associated with allergic inflammation.(17)

Whether or not asthma-like syndrome after preterm birth could be described as an asthma phenotype is a matter of opinion. Anti-asthma drugs are commonly prescribed, though have not yet been proven effective in treating the asthma-like symptoms in this growing group of symptomatic children and adults. It is not known if the longstanding effects on lung function development after preterm birth and in children with the transient early wheeze phenotype share pathophysiological mechanisms, but the bronchial obstructive pattern and absence of airway inflammation in both entities make comparisons reasonable.

2 BACKGROUND

2.1 NORMAL LUNG DEVELOPMENT

By volume, the lungs are the largest organs in the body, but consist of only half a liter of tissue. The structures of the lungs separate approximately half a liter of blood from a certain volume of air, which varies during the course of normal breathing. The configuration of the lungs allows a minimum of tissue to create a fairly stable surface for gas exchange, at nearly the size of a tennis court, inside a limited space (thorax cavity). The human airway tree consists of on average 23 generations of branches. Generation 0 (trachea) to approximately generation 15 are lined with bronchial epithelium and functionally named conducting airways. In the conducting airways, which hold less than a tenth of the resting lung volume, i.e.functional residual capacity, no gas exchange takes place. From airway generations 15 – 16, alveoli with gas-exchanging surfaces start to appear. As branching continues, the alveoli increase in numbers, and in the last generation, alveolar sacs form blind endings.(18) Generations 15 – 23 are called the acinar airways and hold the vast majority of the resting air volume. Functionally, the acinar airways serve as the gas-exchanging zone. Bronchial generations 8/9 to 23 are for practical reasons called peripheral or small airways.(Figure 1)

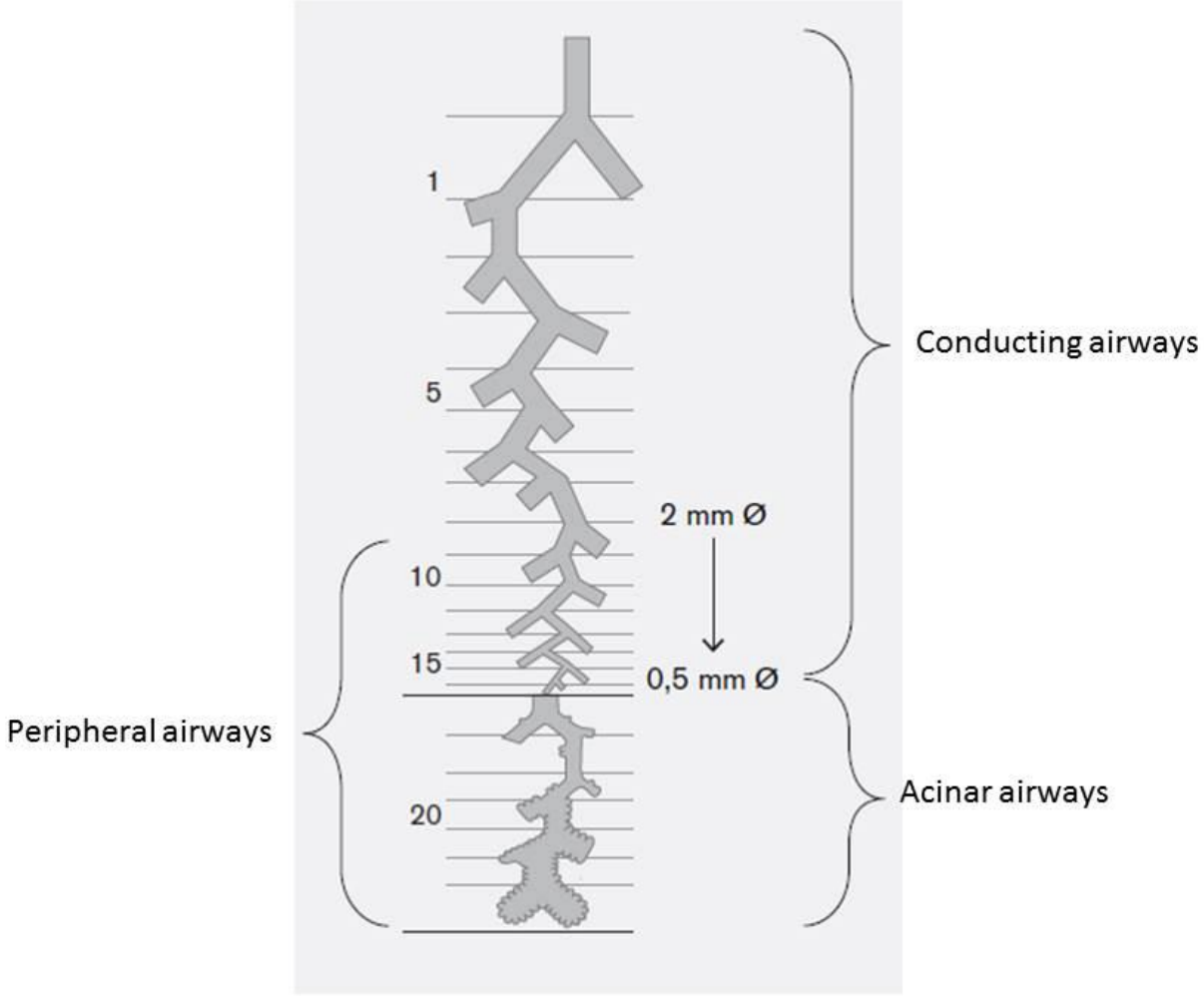

Figure 1. Schematic cartoon of the airway tree (by courtesy of Per Gustafsson).

The prenatal lung development is divided into four characteristic periods. A fifth period, the so called *alveolar period*, continues postnatally.(19) The first prenatal period, *the embryonic period* (weeks 0 – 6), starts with an epithelial tube, derived from the foregut, which branches into the surrounding mesenchyme to form two lung buds. From the mesenchyme, smooth muscle and blood vessels arise, which follow the dichotomous branching taking place during the period. At the end of the stage, the five main lung lobes and 18 lobules are present. During the next period, *the pseudo-glandular period* (weeks 7 – 16), branching of airways and vessels continues and at the end of the stage conducting airways and pre-acinar vascular pattern are completed. *The canalicular period* (weeks 17 – 24) involves further development of distal airways and appearance of rudimentary alveoli. During this stage, the thin air-blood barrier is formed and early secretion of surface-active agents into air spaces occurs, creating pre-conditions for survival ex utero. In *the saccular period* (weeks 24 – 36/40), the last

generation of airways, the alveolar duct and, at the end of the period, the alveoli are formed. True alveoli start to appear from week 32. The pulmonary surfactant system develops, starts to mature and is increasingly active after the 32nd gestational week. The last four to five weeks before term, the alveolar period, is characterized by a large increase of gas-exchanging surface. At birth alveolarization has barely started – numbers ranging from 50 to 150 million alveoli have been suggested – and most of this further formation of alveoli takes place after birth at term.(20) The length of the alveolar period has lately been debated. Previously, alveoli have been thought to stop multiplying at between 2 – 7 years of age.(21) However, recent human studies suggest that the process might continue into adulthood, which completely changes the perspective on alveolar repair.(22, 23) The final numbers of alveoli in adults are in the range of 300 to 800 million depending on lung size and sex.(24) From birth and up to early adulthood (around 22 years of age in men and slightly earlier in females), the lung size increases proportionally with body size and is affected by sex, age and ethnicity.(25) This enormous development is described to represent a 30-fold increase in gas-exchanging surface and at least a doubling of airway diameter and length.

2.2 LUNG FUNCTION AFTER PRETERM BIRTH

Understanding normal lung development is essential in order to comprehend the different responses of the airways to insults during fetal life or after birth. The short version of this section could be that "the outcome of aberrant lung development depends on the type, severity, and duration of insult, and the developmental stage at which it occurs."(20) Another important cornerstone for the understanding is the concept of tracking lung function during growth.(26, 27) This implies that infants born with impaired lung function, due to in utero or extra-uterine insults, will usually remain at a low percentile for measured lung function from childhood to adulthood.

Initially the effects of preterm birth were described in terms of airway epithelial metaplasia, peribronchial fibrosis, vascular smooth muscle hyperplasia and radiologic findings in infants suffering from severe respiratory distress syndrome. This description of the clinical picture after preterm birth was named bronchopulmonary dysplasia (BPD) by Northway et al. in 1967.(28) After advances in peri- and neonatal care over the last decades, this description only applies to a minority of patients today and is now referred to as "old BPD". The "new" BPD seen in children born today is characterized by disrupted alveolar and vascular

growth.(29) The purpose of diagnosing BPD in infants in the neonatal period could be summarized as serving to enable prediction of length of neonatal care, requirement of oxygen therapy, severity of future respiratory morbidity and lung function impairments, and to aid develop of effective preventive and treatment strategies for respiratory syndrome following preterm birth.(30, 31) To define BPD in the neonatal period, the need for prolonged supplemental oxygen delivery during the first period of life was suggested as a surrogate marker for the disease. Several options of this definition of BPD have been proposed over the years. The most commonly accepted definition used in recent studies for infants born before 32 weeks of gestation is based on the need for supplementary O_2 therapy at 28 days of age, and severity is determined at 36 weeks of gestation as follows:

Mild BPD - breathing room air
Moderate BPD - breathing < 30 % oxygen
Severe BPD - need for ≥ 30 % supplementary oxygen and/or CPAP or ventilator use.(32)

Whether or not mild BPD should be included at all in the definition is debated. Guidelines from the Swedish Neonatal Society, published by the National Board of Health and Welfare, define BPD as the need for supplemental oxygen at gestational week 36, at least if an infant is born very preterm.
The feasibility and criticism of use of BPD diagnosis, irrespective of severity levels, as a prognostic tool for future respiratory morbidity will be discussed later. There is little evidence that BPD relates to specific histopathology, whereas preterm birth seems to do so, as presented below.

The pathogenesis of functional abnormalities seen after preterm birth is complex. The main features in lung function at follow-up after preterm birth, not limited to BPD, are increased airway resistance, reduced compliance of the respiratory system, hyperinflation, airway obstruction, and bronchial hyper-responsiveness.(3, 9, 10, 33, 34) Infants born very or extremely preterm are born in the canalicular and early saccular stages of lung development, when the conducting airways are already developed, and thus vulnerable to insults. Airway wall dimensions have been shown to be increased in infants who died from BPD, compared with infants who died from SIDS (sudden infant death syndrome).(35) Structural and histologic studies of airways in surviving infants after preterm birth are, for obvious reasons, not common. In animal models, airway smooth muscle hyperplasia and airway remodeling have been described after hyperoxia.(36) Being born before alveolar formation is thought to

lead to alveolar arrest, resulting in fewer and larger alveoli after preterm birth. This has been shown in infants during the first years of life.(37) However, the arrest after preterm birth is not thought to be permanent.(29) Recently, studies using ^{3}Helium MRI have shown intriguing results that suggest that alveolar development continues both in healthy children and after preterm birth into adulthood.(22, 23) This is supported by Yammine et al., who found normal alveolar compartment function at school age in former pre-terms using the multiple-breath washout technique.(38) They also reported that impairments in convection-dependent airways (peripheral conducting airways) could influence the obstructive pattern seen after preterm birth. Further support for airway wall involvement in pathology after preterm birth was presented by Henschen et al., who concluded that the physical ability of the airways to carry flow differs in children born preterm compared with in those born at term, and that the difference cannot be explained merely by the size of the airways.(39) In conclusion, in spite of some encouraging findings in recent years, all preterm births are associated with altered and reduced lung function. The complex relationship between lung function and structural deficits is thus still only partially understood.

2.3 ASTHMA PHENOTYPES IN EARLY LIFE IN RELATION TO LUNG FUNCTION

It is well recognized that asthma is not a single disease, but many, with large variations in disease course and outcome. From this knowledge, the concept of asthma phenotypes has evolved in the last decades. The purpose of phenotyping is to aid development of preventive strategies and treatment. The term asthma phenotype describes observed properties or developmental traits of the disease. It can encompass a number of biochemical, clinical and physiological measures, which describe the characteristics of the phenotype, without any reference to a particular pathophysiological process. It is likely that most of the airways, from the central to the most peripheral structures, could be involved in asthma, suggesting different pathophysiology behind a common clinical feature. Asthma endotypes describe the underlying pathological mechanisms. An asthma phenotype is not the result of one single endotype, but of several, and conversely a specific endotype could give rise to several phenotypes.(40) This implies that “one size fits all” concepts for asthma treatment and prevention will fail and indicates the need for targeted approaches.

One approach of phenotyping is to use the relationship between asthma course and lung function. Martinez et al. identified three latent asthma phenotypes based on the presence or

absence of respiratory wheeze during the first six years of life; transient early, persistent and late-onset wheeze. They found that transient early wheeze was associated with lower lung function, measured using spirometry soon after birth, and remained lower than that of never wheezers at follow-up in adolescence.(14, 41) Persistent wheezers had maximal expiratory flows, which were initially no different from those of non-wheezers, but lower at follow-up in adolescence. Finally, the late-onset wheezers had lung function similar to that of non-wheezers, both early after birth and at adolescence. When using more complex approaches than a single presenting symptom, other asthma phenotypes have been identified.(17) Nevertheless, latent asthma phenotypes, based on symptoms or biological measures, are retrospective and provide important information on disease, but have limited utility for clinicians as a prognostic tool. In the absence of evidence for symptom-defined asthma phenotypes and discrete pathophysiological processes in children, studies using other lung function techniques that are more sensitive to peripheral airways than spirometry could aid decisions in the clinical setting. Both inert gas washout and the IOS technique – the latter used in our studies – have been associated with asthma control during childhood. Singer et al. showed abnormal acinar ventilation distribution in children with asthma despite normal spirometry.(42) Similarly, Shi et al. found increased frequency dependence of resistance and reactance in uncontrolled subjects as compared with subjects with controlled asthma, despite spirometry findings were not different in asthmatics and healthy controls.(42, 43) These techniques therefore have the potential to be clinical tools for identifying individuals who could benefit from more intense treatment or interventions. However, it is fair to appreciate that lung function only partly represents the features of a clinical asthma phenotype. It is likely that asthma phenotypes in the future will be based on much more complex information than symptoms and lung function, such as genetics, airway inflammation markers and recorded environmental exposures.

2.4 LUNG FUNCTION TESTING

Objective assessment of lung function is an important component in the diagnosis, management and understanding of respiratory disorders. Information on lung function in the growing child yields information concerning:

- Lung growth and development
- Quantitiative measure of impairment (mild, moderate, severe disease)

- Physiological processes involved
- Effects of interventions
- Epidemiologic evaluations of risk factors for disease

There are several components of the airway walls and the lung parenchyma that could be affected in the developing lung. Several methods have been developed over the past century to assess abnormalities of these structures, with a boost of techniques in the last few decades thanks to the rapid development of computer processing. Possible pathophysiological findings in airway obstruction after preterm birth or in early childhood asthma are abnormalities in airway smooth muscle, airway thickness and airway compliance. To assess this in the present thesis, we have used a mixture of methods, applied from infancy to late adolescence.

2.4.1 Infant lung function testing (ILFT)

The first attempts to measure lung function in infants were made in 1890. The first infant plethysmograph was used in the 1960s in London. During the 1980s, techniques to measure passive lung mechanics and maximal expiratory flows were developed. Generally, ILFT is still mainly used in research, while routine clinical use is still considered limited and not yet fully established. Among the limitations are the need for sedation, expensive equipment, a time-consuming procedure requiring intensive training of staff and limited available reference values. In brief, the IFLT protocol in Study 1 consisted of the single breath single occlusion method for measuring respiratory system compliance (C_{so}) by passive lung mechanics, whole body plethysmography for measuring functional residual capacity (FRC), and forced expiratory flow volume loops using the tidal volume rapid thoracic compression (TVRTC) and raised volume rapid thoracic compression (RVRTC) methods. The tests were performed with the subject wearing a face mask over the nose and mouth and lying in the supine position during quiet sleep after sedation with chloral hydrate (60 – 100 mg/kg body weight).

The single occlusion technique to measure compliance of the respiratory system, C_{co}, is based on the ability to invoke the Hering Breuer inflation reflex. If the airway is occluded above FRC, the reflex leads to a period of relaxation of respiratory muscles and a prolonged relaxed expiration.(44) During this brief period, pressures can equilibrate across the respiratory system so that alveolar pressure can be measured at airway opening. C_{so} is assumed to mainly

reflect the elastic recoil pressure of the lung, thus giving information on the elastic properties of the lung. This is due to the highly compliant chest wall in first years of life.

FRC is determined through whole body plethysmography, which measures all gas in the lungs at the end of expiration, including any trapped gas behind closed airways. The basic principal is based on Boyle's law, which states that for a fixed mass, the product of pressure and volume of a gas are constant.(45) Increased values of FRC indicate trapped gas due to intrathoracic airway obstruction and decreased values could in infancy indicate congenital problems or disrupted alveolar development.

Rapid thoracic compression (RTC) method, often referred to as the "squeeze"method, measures flow limitations and is be performed during tidal breathing at the end of inspiration (Tidal Volume RTC, TVRTC) or at the end of a maximal inspiration (Raised Volume RTC, RVRTC).(46) The raised volume is produced by applying a pressure of 30 cm H_2O to the airway and is considered to be equal to a maximal voluntary inspiration. To obtain forced expiratory flows, the infant is made to exhale at a maximal rate by compressing the thorax using an inflatable jacket. Determinates of flow are the elastic recoil of the lung, the chest wall, airway dimensions and airway stability. TVRTC measures flow at resting volume, V_{max}FRC, which means that values reflect either airway properties or lung volume or both. Flow measures by the RVRTC method mimics conventional spirometry and are more closely related to flows measured later during childhood and adulthood. Time flows, such as forced expiratory volume at 0.5 sec, are commonly reported. RVRTC also produces the forced expiratory volume, FVC, maximal expirable volume after a maximal inspiration. This volume, in contrast to FRC, does not include trapped gas.

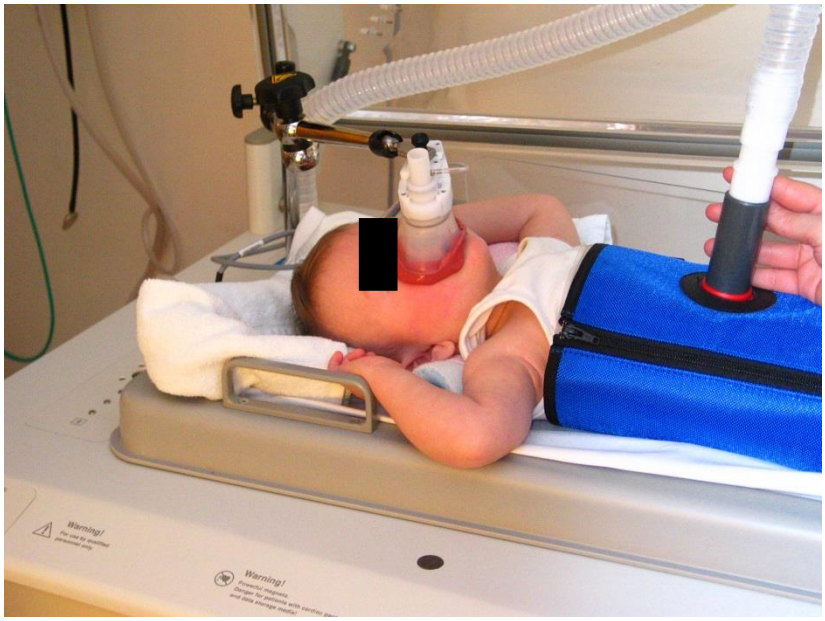

Figure 2. Infant lung function test (photo by P Thunqvist)

No single infant lung function test can on its own be used to fully characterize physiological and mechanical properties; the use of several tests is often indicated.

2.4.2 Dynamic spirometry

Forced expiratory volume measurements in relation to time were first described by Robert Tiffeneau in 1947. Since then, dynamic spirometry has become the most used and established lung function method in both clinical settings and research. There is an extensive, well-recognized body of tutorials and standardization literature on spirometry.(47)

Dynamic spirometry (Figure 3) measures the maximal volumes and flows that can be inhaled and exhaled by an individual. Forced expiratory volume (FVC) is the volume change between a full inspiration and a maximal forced expiration. FVC represents the size of the lungs, but does not include the volume left in the lung after airway closure. Thus, a low value indicates small lungs or air-trapping. To measure the volume left in the lungs after a maximal exhalation either a plethysmographic or a gas-washout technique is required.
The maximal expiratory flow volumes curves are based on the assumption of reaching flow limitation during the maximal expiration maneuver. If increasing expiratory effort is applied to the airway and no further increase of flow is seen, flow limitation has been achieved. A low flow, compared with reference values or healthy control subjects, indicates higher resistance or smaller airway caliber, which is characteristic of airway obstruction. Dynamic spirometry reports volumes at different time points during exhalation, typically at 1 second (FEV_1) in older children and adults and at 0.5 second in infants ($FEV_{0.5}$). Flow is also reported and referenced to a specific volume, typically flow at 50 % of FVC (MEF_{50}). Expired volumes at specific time points, particularly FEV_1, have proven to be robust and less variable than flows at specific volumes and are therefore preferred as a measure of lung function. Finally, the ratio between FEV_1 and FVC (FEV_1/FVC) is reported as a measure of proportionality between airway dimension and lung volumes. A low ratio is seen in airway obstruction and a high ratio indicates a restrictive lung pattern in the presence of low lung volume.

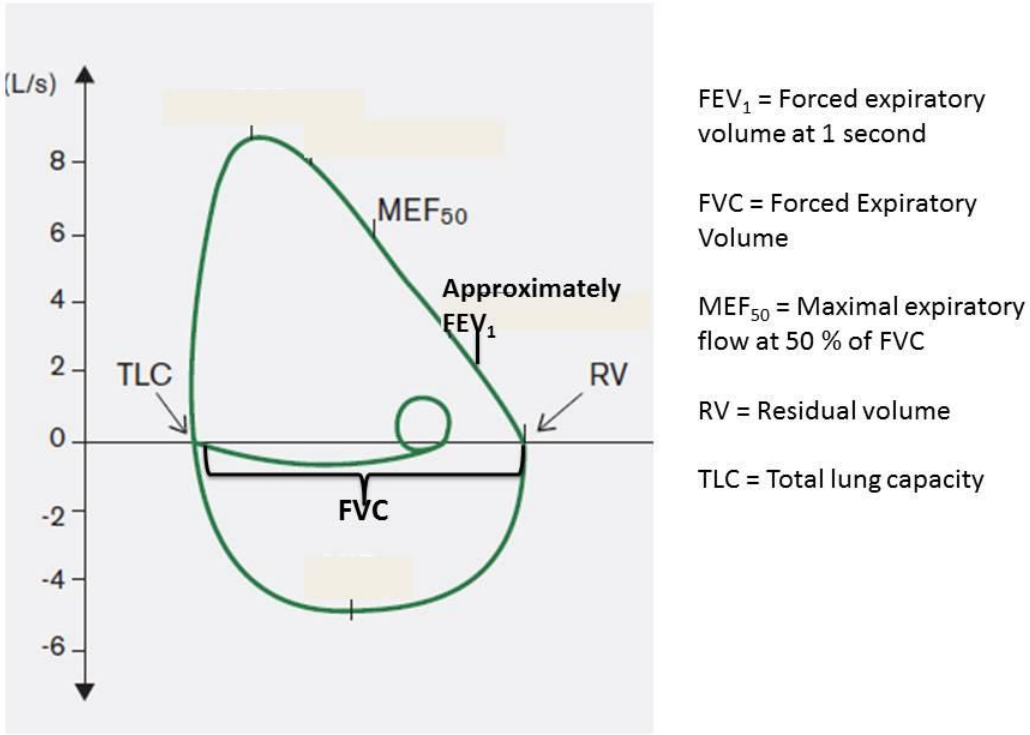

Figure 3. Dynamic spirometric flow volume loop (by courtesy of Per Gustafsson)

2.4.3 Impulse oscillometry (IOS)

IOS is a commercially available forced oscillation technique (FOT). FOT was first described by Dubois in 1956 (interestingly in the same time span as dynamic spirometry).(48) The principal of FOT is that respiratory mechanics can be measured using superimposition of external pressure oscillations to the respiratory system during tidal breathing, which is a very slow oscillatory process. The output, which is a complex relationship of the artificial pressure waves from the loudspeaker and the test subject's tidal breathing waveform, is called respiratory impedance (Z_{rs}) and represents mechanical properties of the respiratory system (rs).(49) The IOS system applies complex sound pulses 5 times per second to the airways. Sophisticated signal processing by Fast Fourier Transform (FFT) analysis used by the software resolves Z_{rs} with respect to frequency of the sound waves, giving a spectrum of Z_{rs} from 5 to 35 Hz, and extrapolates data even down to 3 Hz. Z_{rs} can be separated into its two components resistance (R) and reactance (X). R represents energy loss due to "friction", while X represents energy captured by the respiratory system . X has two components: capacitive reactance, also called capacitance (C), and inertive reactance, also called inertance

(I). Capacitance relates to the energy stored when the walls of the respiratory system, including the airways and the chest wall, are forced to widen in response to the pressure force put on them. Thus, it is related to the elements of the lungs determining compliance. Inertance reflects the energy required to accelerate the air column in the airways, C and I represent opposing forces. At lower frequencies of the sound wave C predominates, while I predominates at higher frequencies. At an intermediate frequency, called the resonant frequency (f_{res}), C and I are balanced and X equals zero. The area under the curve of X from 5Hz to f_{res} is called the area of reactance (AX). It was introduced by the American respiratory physiologist Michael D Goldman and is sometimes referred to as Goldman´s triangle (Figure 4). The use of multiple oscillation frequencies allows a separation of large and smaller airway resistance. Higher frequencies (> 20 Hz) travel shorter distances and can only reach large or intermediate airways. Lower frequencies (< 15 Hz) are transmitted more distally in the lungs. As a result, low frequencies represent small and large airways, and higher frequencies represent only large airways. R, is in healthy adults nearly independent of oscillation frequency (R is equal at low and high Hz). In young children and adults with peripheral airway obstruction, R is higher at low frequencies, compared to high frequencies; this phenomenon is called frequency-dependence of resistance (fdR). In practice, fdR is an index of bronchial obstruction and is expressed as the resistance at 5 Hz less the resistance at 20 Hz (R_{5-20}). Similarly, low frequencies of the sound wave X therefore represents elastic properties in the periphery.

AX correlates with fdR and is used as a sensitive index of peripheral airway obstruction.(50) Forced oscillation techniques have the advantage over spirometry that they minimize demands on the patient, requiring only passive cooperation and making the techniques feasible to children too young to perform spirometry or for other reasons unable to perform a expiratory maneuver.

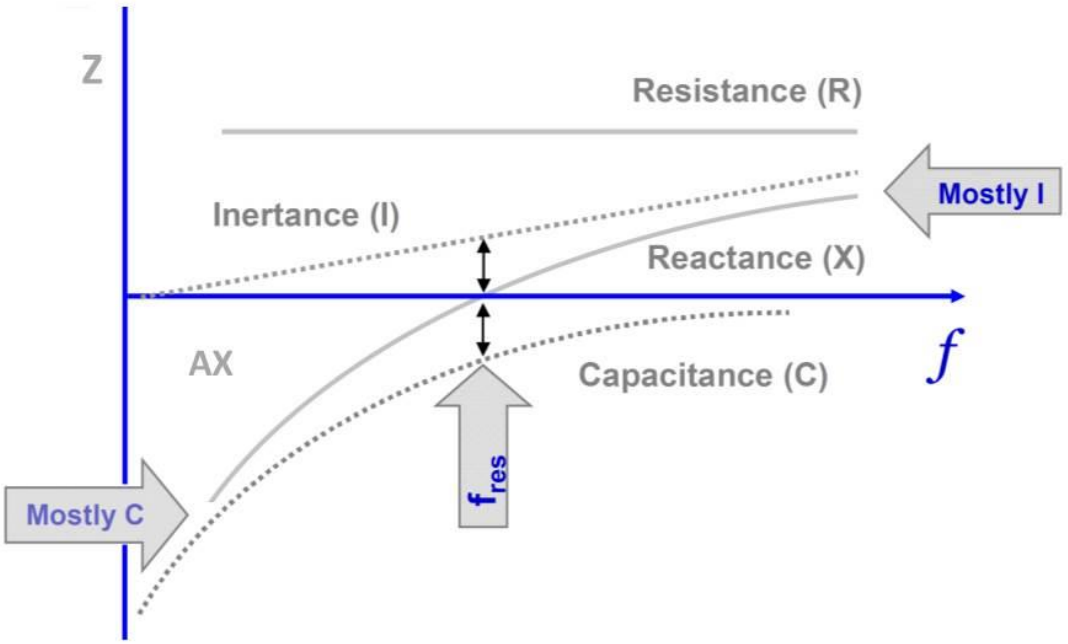

Figure 4. Schematic illustration of components of impedance (Z) in a healthy subject with absent frequency dependence of resistance (by courtesy of Per Gustafsson)

2.5 TREATMENT OF OBSTRUCTIVE DISEASE

Treatment of pediatric asthma

Updated local and international guidelines for treatment of asthma in childhood have been published (Global initiative for asthma, Läkemedelsverket 2015, Barnallergisektionen 2015). However, treatment of early infant wheeze, most often trigged by viral respiratory infections is less evidence based and still widely discussed.(51) As noted above, identifying asthma phenotypes early in life with an increased risk of developing persistent obstructive disease is challenging.(16) In the absence of sensitive and specific predictive algorithms to identify who will and who will not respond to regular asthma treatment, it is likely that a significant proportion of early asthma patients given treatment suggested by guidelines will not respond.(15)

Treatment of asthma-like symptoms after preterm birth

There are no published guidelines available regarding treatment of respiratory symptoms, often described as asthma-like, in children or adults born preterm. In clinical practice, it is common that conventional asthma treatment is used, even though the scientific background is almost non-existing.

Anti-inflammatory treatment for patients born preterm has been investigated in three studies. A four-week crossover study with 400 microgram budesonide daily at 8 years of age in preterm children (mean gestational age 28 weeks) had no effect on respiratory symptoms or airway function during the active period.(52) Pelkonen et al. studied a similar preterm group at 10 years of age. They demonstrated reduced lung function, increased responsiveness to beta2-agonists and/or increased diurnal peak expiratory flow before the start of the study. After 4 months of inhaled corticosteroid therapy (800 microgram budesonide daily) they found no effect on clinical outcome or spirometric values, but some effect on peak expiratory flow (PEF) variation.(53)

Several studies have addressed exercise-induced bronchoconstriction in preterm children and reported significant bronchodilation of beta2-agonist after exercise.(54, 55) However, since none of these studies used treatment before exercise, it remains unclear if asthma treatment has any role in preventing exercise-induced bronchial obstruction.

3 OBJECTIVES

Overall aims

- To determine lung function development from infancy to adolescence in relation to preterm birth and asthma in early childhood.
- To investigate if similarities and differences in measured lung function after preterm birth and among childhood asthma phenotypes may provide information on mechanisms for the obstructive patterns seen in these different conditions.

Specific research questions

- What is the effect of preterm birth on lung function in infancy (Study 1), school age (Study 2), and adolescence (Study 3)?
- What is the relationship between early staging of BPD severity and subseqent measured lung function (Study 1, Study 2)?
- What are the long-term effects on lung function development after preterm birth (Study 1, Study 2, Study 3)?
- What is the association between different childhood asthma phenotypes and lung function (Study 4)?

4 SUBJECTS AND METHODS

4.1 STUDY POPULATIONS AND DESIGN

The study subjects included in the present thesis are participants in one of three different cohorts. The preterm infant study (Study 1) is based on subjects from Sachs' Children and Youth Hospital and represent a longitudinal cohort study of patients recruited 2006 – 2008. Subjects in the early school-age preterm study (Study 2) were recruited from a national birth cohort (EXPRESS), a prospective cohort study of extremely premature children with case-control design. The moderate-to-late preterm study (Study 3) and the early asthma study (Study 4) are based on the prospective birth cohort BAMSE and include longitudinal follow-up data.

4.1.1 Preterm infant study population (Study 1)

During 2006 – 2008, 58 prematurely born children (gestational weeks 23 – 30) diagnosed with BPD and admitted to the Neonatal Units at Sachs' Children and Youth Hospital were recruited to the study. They were consecutively invited to follow-ups, including lung function tests, at 6 and 18 months postnatal age. The parents of 55 infants agreed to participate in the study.

4.1.2 School-age preterm study population (Study 2)

EXPRESS (Extremely Preterm Infants in Sweden Study) included all infants in Sweden born before 27 weeks of gestation between April 1 2004 and March 31 2007.(7, 56) In three out of seven health care regions in Sweden, all participants in the EXPRESS study were invited to a follow-up at 6½ years of age (± 3 months). In total, 250 children (51 % of the total EXPRESS cohort alive at the time of inclusion) were eligible for inclusion. Thirty-four children were lost to follow-up and 38 declined participation, leaving 178 children who were included in the study.

4.1.3 Moderate-to-late preterm (Study 3) and early childhood asthma study populations (Study 4)

The study populations in Studies 3 and 4 are both derived from the BAMSE (Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology) cohort, which is a

prospective birth cohort including 4 089 Swedish children.(58) The plan was initially to establish risk factors for allergy-related disease in childhood up to the age of 4 years.(57) Since then, several follow-ups have been performed and a investigation at 24 years of age is currently being planned. Parents of all infants born between 1994 and 1996 in predefined areas of Stockholm, including inner city, urban and suburban districts were asked to participate in the study. The original cohort consisted of 75 % of eligible children. Exclusion criteria were: the family planned to move within one year of the study start; insufficient knowledge of the Swedish language; the family had a seriously ill child; or an older sister or brother was already included in the study. Data on detailed residential characteristics, environmental factors, and allergic heredity were collected through parental questionnaires when the children were approximately two months of age (time of inclusion). At 1, 2, 4, 8, 12 and 16 years of age, questionnaires focusing on symptoms and medications related to respiratory and allergic diseases were administered, with response rates ranging from 82 – 96 % at each occasion. The participants have been invited to follow-ups that included lung function assessments at three occasions: 4, 8 and 16 years of age. At 4 years of age, 2 599 children successfully took part in testing that included Peak Expiratory Flow (PEF) measures.(59). Acceptable spirometry was performed in 1 832 children at 8 years of age.(60). At 16 years of age, all subjects were invited to follow-ups including both spirometry and IOS. The study samples in Studies 3 and 4 differ somewhat and are described below.

In Study 3, data from questionnaires at 2.5 months (baseline), 8 years and 16 years, and lung function at 8 and 16 years of age were used. After exclusion of children born very and extremely preterm (< gestational week 32, n = 24) and post-term (> gestational week 42, n = 373), 3 692 subjects (90 % of the original BAMSE cohort) were eligible for the study. Of those, 2 782 participated in the 8- and/or 16-year follow-up and were included in the study.

In Study 4, data from questionnaires at 2.5 months (baseline), 1, 2, 4, 8 and 16 years of age and lung function from 8 and 16 years were used. Of the 2 605 subjects participating in the 16-year follow-up, 2 355 (90 %) had sufficient data on wheeze/asthma to be classified into the predefined asthma groups (see below), and were included in the study.

4.2 DEFINITIONS OF STUDY GROUPS

Preterm infant study group definitions (Study 1)

The participants were children born preterm between gestational weeks 23 – 30 and diagnosed as having BPD in the neonatal period. Perinatal and neonatal data were obtained from medical records. The diagnosis of BPD was based on the need for supplementary O_2 therapy at 28 days of age, and severity was determined at 36 weeks of gestation as follows:
Mild BPD - breathing room air.
Moderate/severe BPD - need for supplementary oxygen, at least more than room air and/or CPAP or ventilator use.(32)

School-age preterm group definitions (Study 2)
Children in the preterm group were all born extremely preterm, from week 22 and before week 27. Perinatal and neonatal data were obtained from the EXPRESS study.(7) Bronchopulmonary dysplasia (BPD) in the neonatal period was categorized as either moderate BPD (breathing > 21 % but < 30 % oxygen) or severe BPD (breathing at least 30 % oxygen and/or CPAP) at 36 weeks of postmenstrual age.(32) Small for gestational age (SGA) was defined as a birth weight two standard deviations or more below the mean, and appropriate for gestational age (AGA) was defined as birth weight within two standard deviations of the mean, based on a Swedish sex and gestational age-specific reference for normal fetal growth.(61)
Using the Swedish Medical Birth Register, each preterm child was matched to a randomly selected healthy control born at term (gestational age 37 – 41 weeks). Matched factors were mother's country of birth, date of delivery, hospital of birth and sex.

Moderate-to-late preterm study group definitions (Study 3)
The participants were divided into two groups categorized by gestational age (GA), moderate-to-late preterm (32 – 36 weeks) and term (37 – 41 weeks). GA was obtained from the Swedish Medical Birth Registry or, when registry data was not available, by parental reporting in the baseline questionnaire.

Early childhood asthma study group definitions (Study 4)
The children were separated into symptom groups based on data about asthma and/or wheeze onset and duration from the questionnaires at 1, 2, 4, 8 and 16 years of age. The following definitions were used: 'never asthma' (reference group, did not fulfill any asthma criteria from birth to 16 years of age), 'early transient asthma' (asthma between age 0 and 4 years, no wheeze after), 'early persistent' (asthma in the first 4 years and asthma at 16 years), and 'late-onset asthma' (no asthma at ages 1, 2, or 4, but asthma at age 16).

4.3 DEFINITIONS OF ASTHMA AND RESPIRATORY OUTCOMES

Preterm infant study respiratory outcomes (Study 1)

In order to classify participants into symptom or no-symptom groups, details on ongoing and previous respiratory symptoms, such as recurrent or chronic cough, wheeze, breathing problems and medication for respiratory symptoms, were obtained from parents in follow-ups at 6 and 18 months of age and from hospital-based medical records.

School-age preterm study respiratory outcomes (Study 2)

Information on asthma-like disease, defined as respiratory wheeze in the previous 12 months and/or current use of asthma medication (beta2-agonists, inhaled corticosteroids, antileukotrienes) in the previous 12 months, was obtained through questionnaires filled out at the time of follow-up.

Moderate-to-late preterm study respiratory outcomes (Study 3)

Asthma was defined as fulfilling at least two of the following three criteria; 1) symptoms of wheeze during the 12 months prior to the date of questionnaire, 2) doctor's diagnosed asthma (from birth to the date of the questionnaire), 3) asthma medication taken occasionally or regularly during the 12 months prior to the date of questionnaire.(62) Wheeze was defined as at least one episode of wheeze during the 12 months prior to the date of the questionnaire.

Early childhood asthma study respiratory outcomes (Study 4)

Asthma at age 1 and 2 years was defined as at least 3 episodes of wheezing in the previous 12 months, combined with either inhaled steroid therapy or signs of airway hyper-reactivity (wheezing or severe coughing at excitement or cold weather, or disturbing cough at night) without ongoing cold. For subjects at 4, 8 and 16 years of age, the definition of asthma was at least 3 episodes of wheezing in the previous 12 months, or 1 episode if the child had been given inhaled steroid therapy.(63) Wheeze was defined as one or more episodes of wheezing in the previous 12 months.

4.4 LUNG FUNCTION TESTS

4.4.1 Infant lung function tests in Study 1

Lung function tests were performed with the subject in the supine position during quiet sleep after sedation with chloral hydrate (60 – 100 mg/kg administered orally or rectally) using the Master Screen Baby BodyPlethysmograph (Erich Jaeger AG, Würtzburg, Germany). The

lung function protocol included four types of measurements: Passive lung mechanics measuring compliance of the respiratory system by a single occlusion method (C_{so}),(44) lung volume at functional respiratory capacity (FRC) by whole body plethysmography,(64) maximal flow at FRC, V_{max}FRC from a partial expiratory flow-volume loop (TVRTC) and from raised volume technique (RVRTC) forced expiratory volume (FVC), forced expiratory volume at 0.5 sec ($FEV_{0.5}$) and mid-expiratory flow (MEF_{50}) were obtained.(46) The procedures for the measurements are described in detail elsewhere.(64-66) Briefly, C_{so} results were reported as the highest value from three acceptable recordings and accepted curves had to have a plateau during occlusion of 100 ms and linearity of the flow volume curve during expiration of $r^2 > 0.98$. FRC was measured during at least two end-inspiratory occlusions by whole body plethysmography and calculated from at least two technically acceptable measurements. V_{max}FRC, was reported as the highest technically acceptable recordings, where no further increments of air into the bladder under the non-elastic vest resulted in higher flow. For RVRTC variables, the jacket pressure producing the highest flow during TVRTC was used. Analysis was performed on the values for FVC, $FEV_{0,5}$ and MEF_{50} from the maneuver having the highest sum of $FEV_{0,4}$ and FVC.

4.4.2 Spirometry in Studies 2 – 4.

When subjects were 6½ years (Study 2) and 16 years (Studies 3 and 4), spirometry was performed using the Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, CA, USA).(67) When subjects were 8 years of age (Studies 3 and 4), lung function testing was performed using the 2200 Pulmonary Function Laboratory (Sensormedics, Anaheim, CA, USA). Spirometry was performed according to ATS/ERS criteria at all occasions.(47) The participant performed at least three MEFV (Maximum Expiratory Flow Volume) recordings in the sitting position, wearing a nose clip. The highest values of FVC and FEV_1 were extracted and used for analysis, provided that the participant's effort was evaluated as being maximal by the test leader, the MEFV curve passed visual quality inspection, and provided that the two highest FVC and FEV_1 and MEF_{50} (Studies 2 and 3) readings were reproducible according to the ATS/ERS criteria. FEV1/FVC ratios were expressed as percentages. The spirometry system was calibrated each day using a 3 L precision syringe.

4.4.3 Impulse oscillometry (IOS) in Studies 2 – 4

IOS measurements were performed using Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, CA, USA).(67) The IOS system has been described in detail elsewhere.(49, 68) Briefly, the IOS system generates small pressure oscillations to the airways through a loudspeaker and the software measures respiratory impedance, which

includes the respiratory resistance (R_{rs}) and respiratory reactance (X_{rs}). All IOS measurements were performed during tidal breathing with the child sitting in an upright position, with lips sealed around the mouthpiece and cheeks supported by the hands. For each participant, a minimum of two recordings lasting at least 20 seconds, without artefacts, were saved for later analysis. Quality control was performed at the time of the examination by visual inspection of the waveforms. Recordings with sighs, swallows and coughs were not accepted. A coherence between recordings of > 0.80 at 10 Hz was used as the criterion for an appropriately performed test. The mean value of the resistance at 5 Hertz (R_5), the resistance at 20 Hertz (R_{20}), the frequency dependence of resistance (fdR, i.e. R_{5-20}) and the area under the curve of negative reactance values (AX) were calculated off-line. AX was reported in Study 2. In Studies 3 and 4, $AX^{0.5}$ was used to report reactance instead. AX may boost the assessed response inappropriately compared with the R_{5-20} response, because it multiplies two partly independent reactions of the airways. We therefore "linearized" AX in Studies 3 and 4 by reporting its square root as well ("$AX^{0.5}$"). The IOS system was calibrated each day with a reference resistance of 0.20 $kPa \cdot L^{-1} \cdot s^{-1}$.

4.4.4 Measures of bronchodilator response in Study 3

Pulmonary function after bronchodilation was measured using both methods (spirometry, IOS as described above), approximately 20 minutes after an inhaled dose of salbutamol (4 x 100 microgram Airomir®, Teva) through a valved spacer device.

4.5 STATISTICAL METHODS

Analyses in all studies were performed with the Stata 12.1 software package (StataCorp LP, College Station, TX, USA).

In all four presented studies, a p-value < 0.05 was considered to be statistically significant.

Infant preterm study statistics (Study 1)

Demographic, perinatal data and differences in symptoms were compared between groups (BPD severity groups, symptom groups) of children using Student's t-tests or the Pearson chi-square test. Each individual lung function test result was converted to a z-score using published reference equations.(69-71) Abnormal lung function was defined as a z-score lower than 1.96. For comparison with other studies, C_{so} and FRC were also reported as units per kg body weight. Differences in lung function according to BPD severity or symptoms were assessed using the Mann–Whitney rank sum test. Lung function change over time was

assessed by comparing z-scores between 6 and 18 months using the Wilcoxon matched-pairs signed-rank test.

School-age preterm study statistics (Study 2)

Demographic and perinatal data are reported as means and standard deviations, ranges or proportions and percentages. Comparisons between the preterm and control groups were performed using the Student's t-test or the Pearson chi-square test. Lung function in the different groups of children was assessed using multiple linear regression analyses, adjusting group comparisons for age and height at the time of measurement, examination site and sex. FVC, FEV_1 and $FEV_{0.75}$ were converted to z-scores using the Global Lung Initiative reference values (GLI).(25) The lower limit of normal (LLN) was defined as z-scores for FEV_1 below -1.64 (< 5^{th} percentile) and -1.96 (< 2.5^{th} percentile),(25) respectively, and the odds for children born preterm having a FEV_1 below these LLN values were calculated using logistic regression analysis.

Moderate-to-late preterm study and Early childhood asthma study statistics (Studies 3 – 4)

Demographic data, mean height and weight at time of examination were compared between groups of children using Student's t-tests or the Pearson chi-square test. In Study 3, the associations between term/preterm groups and spirometry variables were assessed through linear regression analysis and IOS variables were assessed through quantile regression analysis, for males and females separately, adjusting for age and height at the time of measurement. IOS variables were also adjusted for maternal smoking. Since the distribution of the values of IOS was skewed to the right we utilized quantile regression (72, 73), also called regression on the median, to estimate associations between groups and IOS variables. In Study 4, the associations between asthma groups and lung function (spirometry/IOS) variables were assessed using linear regression analysis adjusted for age, height, weight and sex. In Study 4, we also performed quantile regression on the median for IOS data.

In Study 3, associations between preterm birth and z-scores of FEV_1 according to the Global Lung Initiative reference values (GLI) were also evaluated.(25)

To make full use of the repeated data sampling, a longitudinal analysis with mixed models was used in both studies. Time-dependent covariates included in the model were height and age in Study 3, and in Study 4 weight was also included. Fixed covariates were, in Study 3, term/preterm birth group, sex, and maternal smoking, and in Study 4 asthma group and sex. To assess potential different effects for the change over time, a preterm/term group-by-time

interaction term was used in Study 3 and an interaction term between the time and asthma group was included in the model in Study 4.

4.6 ETHICAL APPROVALS

Approvals were obtained from the Regional Ethical Review Board in Stockholm for all four studies: Preterm infant study (Study 1); 457/03, 6½ years preterm study (Study 2); 2010/520-31/2 and amendment 2011/376-32, and the two BAMSE cohort-based studies (Studies 3 and 4); 93:189 and amendments 02-420 and 210/1474-31/3.

5 RESULTS

5.1 LUNG FUNCTION AT 6 AND 18 MONTHS AFTER PRETERM BIRTH: PRETERM INFANT STUDY (1)

An overview of the participants at 6 and 18 months of age is presented in figure 5.

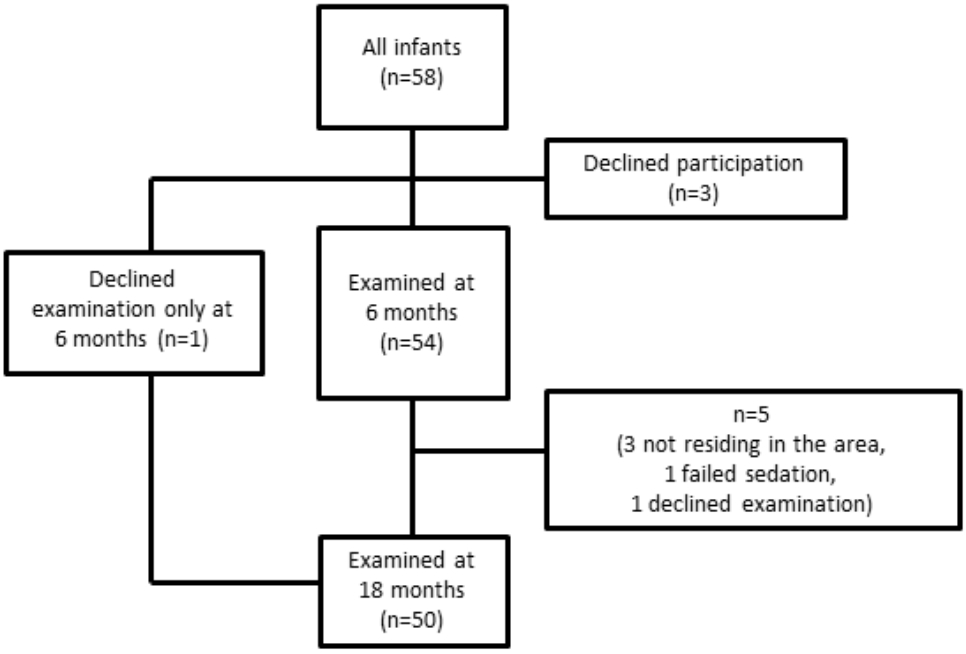

Fig. 5. Overview of infants who met inclusion criteria and their participation during the study.

Maximal forced expiratory flows were low, defined as below -1.96 z-scores, for the majority of children at both 6 and 18 months, except for $V_{max}FRC$ at 6 months of age.
C_{so} was the only variable that differed statistically between BPD groups, where the moderate/severe BPD group presented with lower values than the mild BPD group at both time points, even though C_{so} was within normal limits for both BPD severity groups (except for moderate/severe BPD at age 6 months). The symptomatic group had significantly lower values for C_{so}, $V_{max}FRC$ and MEF_{50} at both 6 and 18 months compared to infants without respiratory symptoms (Table 1).
Lung function development over time stratified by BPD group showed that z-scores for $V_{max}FRC$ significantly decreased for both groups.

Table 1 – Comparison of lung function expressed as z-scores at age 6 and 18 months in infants without or with reported symptoms.

	No reported symptoms			Reported symptoms			P-value comparing symptom groups
	n	median	range	n	median	range	
6 months							
$V_{max}FRC$ (z-score)	34	-1.50	-3.38;0.51	12	-2.23	-3.37;-0.88	0.006
FVC (z-score)	26	-0.97	-3.33:1.47	8	-1.24	-3.50;-0.86	0.155
$FEV_{0.5}$ (z-score)	26	-2.33	-4.17;-0.24	8	-2.61	-5.31;-1.31	0.239
MEF_{50} (z-score)	24	-2.15	-4.71;0.28	7	-3.87	-5.18;-2.87	0.006
FRC (z-score)	35	-1.01	-2.64;0.98	15	-0.80	-2.12;1.26	0.325
C_{so} (z-score)	27	-1.63	-3.32;0.10	16	-2.8	-4.41;-0.33	0.004
18 months							
$V_{max}FRC$ (z-score)	22	-1.97	-3.34;-0.14	21	-2.69	-4.33;-1.45	0.001
FVC (z-score)	20	-1.92	-2.52;0.64	18	-2.11	-4.79;-0.53	0.152
$FEV_{0.5}$ (z-score)	19	-2.14	-4.37;-1.32	17	-2.77	-5.02;-1.41	0.132
MEF_{50} (z-score)	21	-1.74	-5.33;-0.55	20	-2.97	-5.58;-0.93	0.048
FRC (z-score)	23	0.05	-1.74;2.16	18	-0.05	-1.45;2.75	0.431
C_{rso} (z-score)	19	-0.44	-2.0;1.36	15	-1.23	-2.86;0.19	0.015

5.2 LUNG FUNCTION AT 6½ YEARS: SCHOOL-AGE PRETERM STUDY (2)

In total, 89 % of the children contributed with any lung function assessment data and the success rates were 87 % for IOS and 54 % for spirometry, Figure 6.

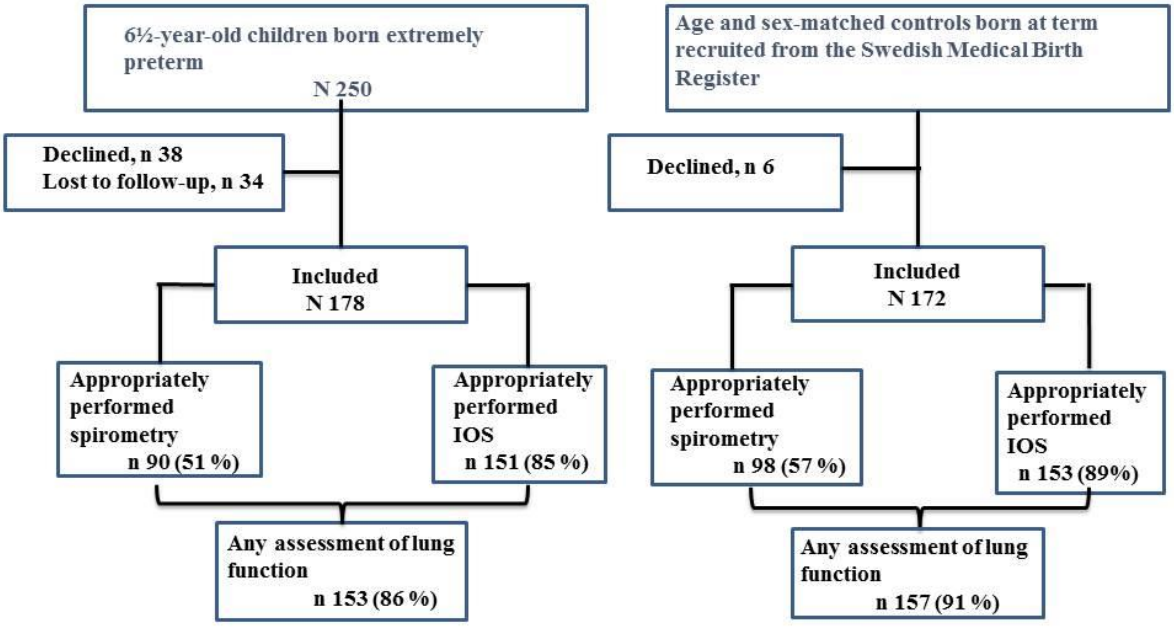

Figure 6. Overview of the preterm children and controls at 6½ years of age.

Of the extremely prematurely born children, 14 % were born small for gestational age (SGA), and 90 % had been diagnosed with either moderate BPD (85 %) or severe BPD (15 %) in the neonatal period. Current wheeze or asthma medication was reported by 40 % of the preterm group and in 15 % of term controls.

Lung function in children born extremely preterm

Extreme preterm birth was associated with significantly lower values for all measured spirometric outcomes and significantly higher values of resistance and reactance measured using IOS, in comparison with controls. Significant reversibility of FEV_1 ($\geq$ 12 %, approximately 20 minutes after an inhaled dose of 400 micrograms of salbutamol through a valved spacer device) was seen in 28 % of preterm children and 10 % of controls. FVC was

below the 5th percentile (lower limit of normal) of the normal population in 14 % of the preterm children and FEV_1 was below the 5th percentile in 23 %.

Gestational age and lung function in children born extremely preterm

Children born at 22 – 24 weeks of gestation had significantly lower values for all measured spirometric outcomes than children born at 25 – 26 weeks of gestation. The proportion of children born at 22 – 24 weeks with lung function below the lower limit of normal was 24 % for FVC and 44 % for FEV_1. IOS results did not differ significantly between the two gestational age groups.

Lung function and asthma-like disease

Among the extremely preterm children, there was an association between reduced z-score for FVC (-0.6, 95 % CI -1.0;-0.1), FEV_1 (-0.8, 95 % CI -1.3;-0.3), increased AX (0.6, 95 % CI 0.1;1.6) and reported asthma-like disease in the previous 12 months (unpublished results).

Lung function in preterm-children born SGA and AGA

Lung function did not differ statistically between children born extremely preterm and SGA and those born AGA, except for a higher frequency dependence of resistance (R_{5-20}) among children born SGA.

Lung function in preterm children and association to severity of BPD

There were no statistically significant lung function differences between the two levels of BPD, except for z-scores for lower ratio FEV_1/FVC in preterm children with severe BPD compared with those with moderate BPD.

5.3 LUNG FUNCTION AT 16 YEARS AFTER MODERATE-TO-LATE PRETERM BIRTH (3)

Of the 4 089 children in the original BAMSE cohort, 2 620 (64 %) and 2 605 (64 %) participated in the 8- and 16-year follow-ups, respectively. The participants and number of successful lung function tests in moderate-late-preterm and term groups are presented in Figure 7.

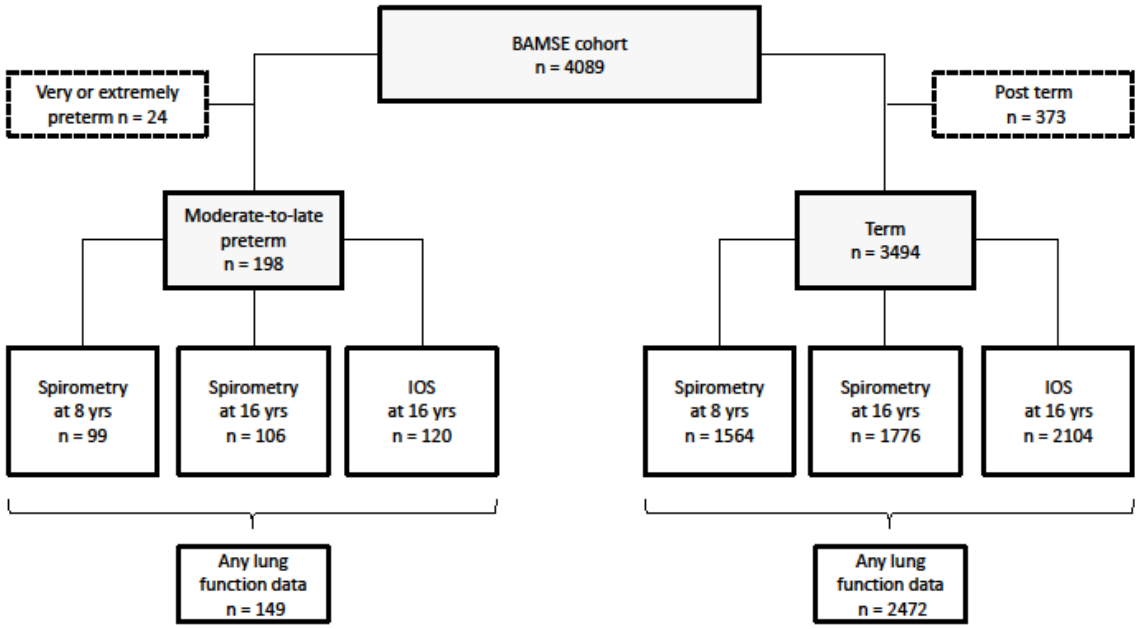

Figure 7. Overview of the participants in the original BAMSE cohort and number of performed and accepted lung function tests at 8 and 16 years used for analysis.

Lung function at 16 years

Among subjects aged 16 years, negative associations between preterm birth and all spirometric indices, except FVC, were observed for both sexes (Table 2). In males, the

moderate-to-late preterm group demonstrated a FEV_1 reduction of 4.0 % compared with term controls, and in females the reduction was 3.4 %.

The IOS results in subjects aged 16 showed significantly higher estimated medians for R_5, R_{5-20}, and $AX^{0.5}$ in moderate-to-late preterm males compared with term males. Although a similar trend was seen for females, a significant difference was only seen for R_5 (Table 2).

To elucidate if the effect on lung function remained in those born closest to term, analysis was restricted to those born at 35 to 36 weeks of gestation. Differences compared with children born at term remained for spirometry results at age 16 (FEV_1 -112 ml, 95 % CI -217.6;-22.3) (unpublished results).

Lung function from age 8 to 16 years

There was an increasingly negative trend over time, between 8 and 16 years of age, for FEV_1 in preterm males compared with term males.

Table 2. Differences in adjusted lung function between moderate-to-late preterm and term groups (adjusting for maternal smoking during pregnancy, height and age at examination).

	Females			Males		
	Diff.	95 % CI	P	Diff.	95 % CI	P
Spirometry at 16 years						
FEV_1 (ml)	-116	-212;-20	0.02	-177	-329;-25	0.02
FEV_1 z-score*	-0.29	-0.52;-0.05	0.02	-0.33	-0.61;-0.05	0.02
MEF_{50} (ml/s)	-409	-660;-159	0.001	-516	-879;-153	0.005
FVC (ml)	-6	-118;106	0.91	-65	-237;106	0.45
FEV_1/FVC (%)	-2.9	-4.5;-1.3	< 0.001	-2.5	-4.4;-0.6	0.01
IOS at 16 years						
R_5 ($Pa \cdot L^{-1} \cdot s^{-1}$)	31.3	6.3;56.3	0.014	34.9	12.0;57.7	0.003
R_{20} ($Pa \cdot L^{-1} \cdot s^{-1}$)	11.2	-9.7;32.1	0.292	15.6	-4.5;35.8	0.129
R_{5-20} ($Pa \cdot L^{-1} \cdot s^{-1}$)	7.0	-4.8;18.8	0.243	20.9	9.8;31.9	< 0.001
$AX^{0.5}$ ($Pa \cdot L^{-1} \cdot s^{-1}$) $^{0.5}$	28.4	-10.6;67.4	0.154	49.9	16.8;83.1	0.003

*Z-score according to GLI 2012(25). Z-score was adjusted for maternal smoking during pregnancy. Diff = difference.

5.4 ASTHMA PHENOTYPES AND LUNG FUNCTION UP TO 16 YEARS OF AGE: EARLY CHILDHOOD ASTHMA STUDY (4)

Children and adolescents taking part in the BAMSE study were at 16 years of age categorized into asthma groups at 16 years as follows: never asthma group, 1 928 individuals (82 % of the

eligible population); early transient asthma, 139 individuals (6 %); early persistent asthma, 61 individuals (3 %); and late-onset asthma, 109 individuals (5 %).

Asthma and spirometry at 16 years

All asthma groups were associated with lower FEV_1, FEV_1/FVC and FEF_{50} values in comparison with the never asthma group at 16 years of age. The lowest values were seen for the early persistent group.

Asthma and impulse oscillometry (IOS) at 16 years

All asthma groups were associated with higher estimated means for R_5 and R_{20} than the never asthma group. Figure 4 shows all percentiles for R_{5-20}, an index indicating heterogeneity in resistance distribution across the airway system. The distribution of R_{5-20} values was similar in the never asthma and transient asthma groups. The persistent asthma group showed a heterogeneous pattern including a wide range of R_{5-20} values and a skewed distribution with extremely large R_{5-20} values.

Asthma and lung function change between 8 and 16 years

We observed significantly less increase in FEV_1 between 8 and 16 years for the early persistent and late-onset groups, but not for the early transient asthma group, as compared with the reference group.

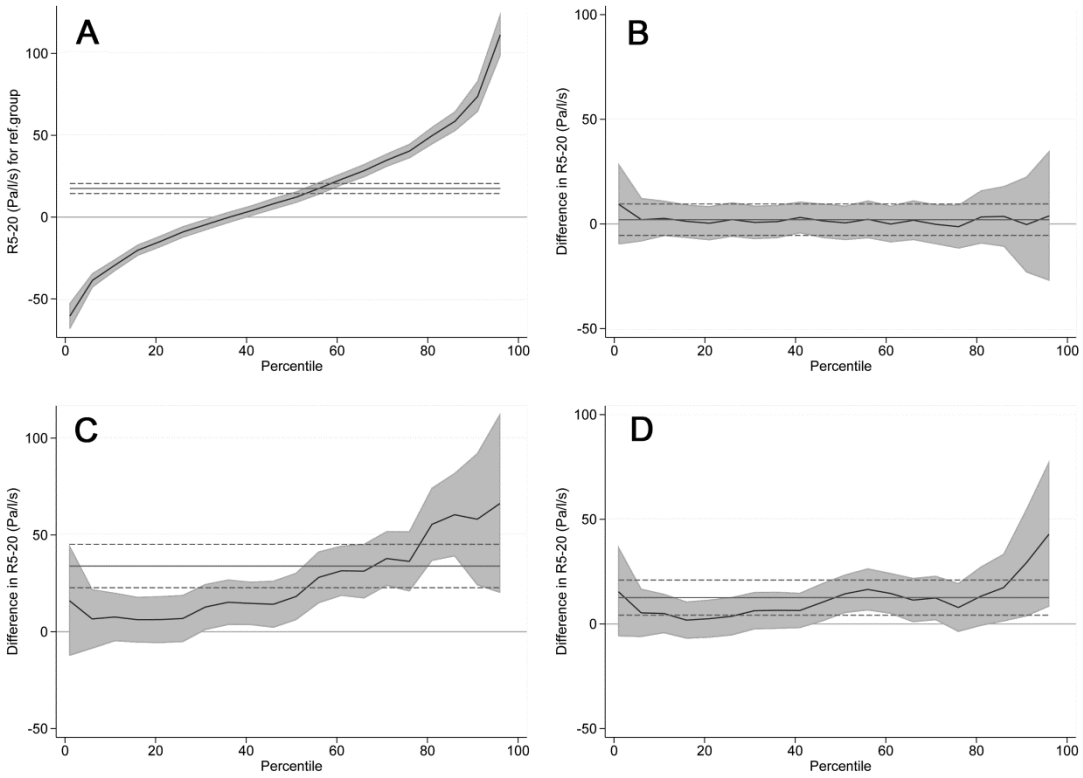

Figure 4. Distribution of R_{5-20} values for the never asthma group (A) and difference between each asthma group and the "never asthma" group at all percentiles (B-D). Estimates at each percentile are shown as solid black lines with 95 percent confidence bands (shaded gray areas). The mean value (solid) and 95 percent confidence intervals (dashed) are shown as parallel black lines.

6 DISCUSSION

6.1 LEVEL OF LUNG FUNCTION AFTER PRETERM BIRTH

6.1.1 Maximal expiratory flows after preterm birth

In Study 1, we found that patients at 6 and 18 months of age with either mild or moderate/severe BPD demonstrated low (< -1.96 z-scores) mean values for $V_{max}FRC$, $FEV_{0.5}$, and MEF_{50}, with the exception of $V_{max}FRC$ at age 18 months for the mild BPD group. The vast majority of preterm infants had clinically significant (< -1.96 z-scores) reduced expiratory flows at 6 and 18 months of age.

Similar infant lung function techniques have been used in other previous studies. Tidal RTC was used at approximately one year of age in a group of children born preterm (gestational weeks 29 – 36) without BPD and a reduction of $V_{max}FRC$ of -2.0 z-score was found.(74) Two other studies show similar reductions of $V_{max}FRC$, -1.79 (40 % below -1.96 z-score) to -2.2 z-score in moderate to severe BPD (75, 76) at 15 – 18 months of age. Filbrun et al. reported that $FEV_{0.5}$ was on average -1.94 z-score at a mean age of 18 months in children with moderate to severe BPD.(77) Recently, infants with mild to severe BPD were shown to have $FEV_{0.5}$ with 2.1 lower z-score at corrected age of 18 months of age as compared with published reference values.(78)

In Study 2, we examined children at 6½ years of age with a history of moderate-to-severe BPD and found that 23 % of preterm children had FEV_1 below the 5th percentile (-1.64 z-score). Among children born in gestational weeks 22 – 24, 44 % had values below the 5th percentile. The EPICure study investigated a similar group as our study. They reported an average reduction of -1.7 z-scores for FEV_1 at 11 years of age in children earlier diagnosed with moderate-to-severe BPD.(33)

In Study 3, we report on reduced maximal expiratory flows at 16 years of age in both females and males born moderate-to-late preterm (gestational weeks 32 – 36). The proportion of individuals with a FEV_1 below the lower limit of normal (< -1.64 z-score) was, in males, significantly larger in the moderate-to-late preterm group than in the term group. Reports on lung function in adolescence in subjects born moderate-to-late preterm are sparse. Kotecha et al. found lower mid-expiratory flows and FEV_1/FVC ratio at 14 – 17 years of age in the subgroup born in gestational weeks 33 – 34, but no difference compared with the term group

for the subgroup born in gestational weeks 35 – 36.(34) We also tested the effect on lung function in those born closest to term by restricting analysis to those born at 35 through 36 weeks of gestation and found that differences compared with children born at term remained for spirometry results.

In summary, in Study 1 and Study 2 we found clinically significant reductions of maximal expiratory flows in infancy and early school age and the levels of reductions are similar to findings in other studies. In Study 3, we found a 3 – 4 % reduction of FEV_1 and more individuals than expected having values below the lower limit of normal after moderate-to-late preterm birth. To our knowledge, Study 2 is the first to report on lung function results associated with the level of extreme preterm birth, showing that within the group of children born extremely preterm, the length of pregnancy matters for lung function development in childhood.

To clarify the possible clinical implication of the lung function reduction after preterm birth, a comparison with natural age-related decline is presented. Natural age-related decline of FEV_1 in adulthood has been calculated to be on average 21 ml/year in females and 27 ml/year in males.(79) The difference measured, for the whole preterm group at 6½ years (330 ml) in Study 2, would correspond to approximately 12 – 15 years of preterm natural lung function decline, depending on sex. Further, if tracking of lung function is assumed, the estimated reduction difference of -1.2 z-score at 6½ years in Study 2 equals 650 ml in an adult male (180 cm), suggesting approximately 24 years of expected preterm decline. Using the same assumption after moderate-to-late preterm birth, the low FEV_1 at 16 years (177 ml) in moderate-to-late preterm males corresponds to a natural decline in adulthood of 7 years. If these predictions have a corresponding predictive value on life expectancy, can only be determined in long-term follow-up studies.

6.1.2 Lung volumes after preterm birth

In Study 1, plethysmographically measured FRC values were within normal range at both 6 and 18 months of age. FVC, measured using raised RTC, was on average lower or borderline lower than expected at 18 months of age. Hofhuis et al. found FRC_{pleth} to be within normal range (-0.6 z-score) in moderate-to-severe BPD at approximately 15 months of age.(76) Fakhoury et al. related FRC to ml per body weight (suggested normal range 16 – 26 ml/kg) and reported normal values at similar ages.(75) However, lung volumes measured using the gas dilution technique (Helium) showed lower FRC_{He} in children with BPD compared with

non-BPD or healthy preterm.(80, 81) Hilgendorf et al. showed that FRC_{pleth} was reduced near term in children with BPD and further reduced after administration of beta2-agonist, suggesting over-inflation after preterm birth.(82) A study combining gas dilution techniques and whole body plethysmography in the same infants reported higher FRC_{pleth} than FRC_{gas} in follow-up of infants with a history of BPD, which also suggested over-inflation and gas trapping in infants with BPD.(83) This notion is supported by results in a study that used raised RTC and whole body plethysmography to calculate fractional lung volumes (TLC and RV) at approximately 18 months of age.(77) They found FVC and TLC to be normal low, but the RV/TLC ratio to be high.
Since FRC is dynamically maintained in infants with obstructed airways, as could be assumed to be the case in infants with BPD, plethysmographically measured FRC may remain normal-to-high. Hence, it is possible that FRC measured plethysmographically, as in Study 1, cannot reflect trapped gas in the premature child, thus overestimating lung volume due to an obstructive pattern.

In Study 2, the reduction of the measured volume among preterm children (gestational weeks 22 – 26) was modest but significant compared with controls (term). Follow-up at school age has shown varying results for FVC in other studies. The EPICure study, which included a cohort with similar level of preterm birth as that in Study 2, found similar significant reduction of FVC, -0.8 z-score.(84) Studies that included a wider range of preterm birth subjects showed no significant differences in FVC between the preterm and term groups.(85-87) However, normal TLC and increased RV/TLC ratio was seen in those studies, indicating an obstructive pattern.

In Study 3, the children born moderate-to-late preterm had normal FVC values, no different to term controls at 16 years of age. This supports findings by other groups.(34, 88)

In summary, lung volumes after preterm birth are normal or normal low and reported reduction in FVC is more likely to be a sign of airway collapsibility during exhalation than pulmonary restrictivity in children born very or extremely preterm. A possible explanation for this functional pattern could be loss of elastic recoil, altered airway wall mechanics and altered alveolarization in early life seen after preterm birth.

6.2 BPD SEVERITY IN RELATION TO LUNG FUNCTION

The definition of BPD published in 2001 based on the NICHD/NHLBI workshop suggests three levels of severity for BPD: mild, moderate/severe and severe disease.(32) In the neonatal period, the grades of BPD severity have been found to be associated with neonatal comorbidities, longer hospital stay and higher gestational age at discharge, showing the usefulness of this definition.(89) A validation of the definition found an association to more severe disease and the use of pulmonary medication at 18 – 22 months of age.(90) Most studies on lung function after preterm birth compare the different definitions of BPD with individuals born at term or non-BPD and do not investigate the effect of BPD severity. Generally, those studies confirm significantly reduced lung function from infancy to adulthood among those with BPD diagnosed in the neonatal period.(9, 84, 91, 92)

We used the consensus definition for BPD severity in Study 1 and Study 2. In Study 1, the only significant difference in lung function at 6 and 18 months of age was lower C_{so} in the moderate/severe group compared with the mild BPD group. Overall, maximal expiratory flows measured using RTC-technique were low (< -1.96 z-scores) for both groups at both time points, with the exception for $V_{max}FRC$ at age 18 months for the mild BPD group.

In Study 2, we compare lung function between moderate and severe BPD groups. Mild BPD was not considered in Study 2, due to the low incidence of mild BPD. Except for lower FEV_1/FVC z-scores in children diagnosed in the neonatal period with severe BPD than in those diagnosed with moderate BPD, lung function at 6½ years of age did not vary in relation to severity of neonatal BPD. This was true for both spirometric and IOS variables. However, we found significant associations between gestational age and reduced lung function measured using spirometry, but not using IOS. Studies investigating lung function at follow-up show conflicting results in relation to BPD severity. Hjalmarson et al. found lower FRC and less efficient gas mixing in severe BPD compared with less severe forms at 9 – 10 months of age. A study from our hospital performed a follow-up at 6 – 8 years of age after preterm birth, showed significantly lower lung function in severe BPD compared with mild/moderate disease.(93) Large differences in percent of predicted FEV_1 were reported between mild and more severe BPD groups at follow-up at 11 – 17 years of age in a cohort of children born in the 1980s and treated at a large tertiary center.(94) Results from a study using RTC methods during the first year of life indicate a non-significant association to BPD severity, in agreement with our findings.(95) A meta-analysis including follow-up studies of

subjects born between 1964 and 2000 at 24 – 36 weeks of gestation reported average FEV_1 reductions of 16 % associated with mild BPD and 19 % for moderate to severe BPD.(3) The lack of clear differences in lung function at follow-up in relation to BPD severity in Study 1 and Study 2 could be due to the fact that all children in both studies were very or extremely prematurely born, thus all having altered alveolarization due to prematurity. In fact, the groups in Study 1 were similar in regards to most background variables. This could indicate limited prognostic utility for BPD diagnose in this respect due to multifactorial etiology and difficulties in early identification of the disease. A limitation of the diagnostic criterion of BPD and BPD severity is the need of supplemental oxygen as a proxy for disease severity. Walsh et al. have reported a physiologic definition of BPD by a standardized timed room-air challenge (96, 97) that is recommended in Sweden since 2014 by the National board of Health and Welfare. However, the length of time for supplemental oxygen therapy and targeted oxygen saturation level could be a function of the immaturity of the child or of the local policies of individual units as reflective of lung pathology during the time of our studies. Further, mode of oxygen delivery, medications and possible upper airway obstruction may also influence gas exchange. It could be questioned if the histopathology of BPD, which the surrogate marker of oxygen need is thought to indicate, is not dichotomous but rather a continuous graded condition. Long-term pulmonary outcomes and supplemental oxygen at post menstrual age of 36 weeks have shown low values of sensitivity and specificity in some studies and might therefore be falsely related.(90, 98) Finally, even the most conservative definition of BPD, supplemental oxygen 28 days before gestational week 36 or at day 28, has been shown to be insufficient as a predictor due to low sensitivity for long-term pulmonary outcome.(93, 99)

In summary, our results suggest that early BPD classification provides limited information on future lung function after preterm birth and other specific risk factors and mechanism for lung function development should be sought. To what extent early measured lung function could contribute needs further investigations.

6.3 LONGITUDINAL ANALYSIS OF EXPIRATORY FLOWS AFTER PRETERM BIRTH AND EARLY BRONCHIAL OBSTRUCTIVE SYMPTOMS

In Study 1, measurements of maximal expiratory flows showed persistently reduced values without catch-up from 6 to 18 months of age for both BPD and non-BPD groups. In fact,

V_{max}FRC measured using tidal RTC decreased significantly at follow-up compared with reference values, while $FEV_{0.5}$ remained at the same level of reduction.
Serial measurements have been performed in a number of studies during the first years of life after preterm birth. A decline of V_{max}FRC was also seen during the first year in children with moderate-to-severe BPD and in healthy children born preterm.(74, 76) Fakhoury et al. performed measurements at 6, 12 and 24 months and reported no change over time in mean z-score V_{max}FRC in children with moderate-to-severe BPD.(75) The raised RTC technique expiratory flows in healthy preterm and children with BPD were low compared with term controls and no catch-up of lung function was seen in any of these three studies during infancy or early childhood.(77, 78, 100)

Generally, these findings during infancy in our studies and others support the notion of tracking of lung function seen in older children and adolescents after very and extreme preterm birth.(92, 101, 102) The difference in development of variables from the tidal volume and raised volume RTC seen in our studies and others could indicate that V_{max}FRC and $FEV_{0.5}$ do not represent the same airway impairment. However, it is also possible that the difference is attributable to the reference values used, as suggested by Lum et al.(103)

In Study 3, moderate-to-late preterm females and males showed no catch-up growth of lung function from 8 to 16 years of age. Instead, the negative effects were augmented between age 8 and 16 years in males. Only two previous studies have longitudinally addressed lung function development after moderate-to-late preterm birth into school age and adolescence. Kotecha et al. found catch-up of FEV_1 from 8 – 9 to 14 – 17 years of age in the subgroup of children born in gestational weeks 33 – 34.(34) A follow-up by Narang et al. from mid-childhood (7 – 9 years) into young adulthood stated that there was no evidence of airway obstruction at 21 years of age in a cohort including participants born in gestational weeks 27 – 37 who, at 7 – 9 years of age, had demonstrated reduced $FEV_{0.75}$.(88) In a meta-analysis that included 24 birth cohorts, particularly late preterm, lung function was negatively associated with preterm birth across the full range of prematurity (< 37 weeks).(104)

In Study 4, longitudinal lung function was compared from 8 to 16 years of age between asthma groups and the never asthma group. The two active asthma groups (early persistent and late-onset asthma) had significantly lower increases of FEV_1. This was seen to a lesser extent in those with early transient asthma and reached significance only for mid-expiratory flows (MEF_{50}). Similar findings were seen in a Norwegian study also identifying both active

asthma and asthma in remission to be associated with less increase of FEV_1 during adolescence.(105) The Melbourne study found a negative lung function development from 12 to 18 years for active asthma (early persistent and late-onset), but not for early transient asthma, which was suggested to be a benign disorder.(106)

In summary, Study 1, Study 3 and Study 4 support the recognized concept of lung function tracking from birth to adolescence.(26) There were no catch-up of lung function seen in the first two years of life after very and extreme preterm birth, or from 8 to 16 years after moderate-to-late preterm birth or in asthma phenotypes during that same age span. In fact, the difference in lung function compared with controls was augmented after moderate-to-late preterm birth and in active asthma suggesting a "two-hit" model including an insult early in life and ongoing processes. Decreasing FEV_1/FVC ratios in young adults after very and extreme preterm birth seen in two other studies (92, 102), particularly in BPD, indicate that similar processes might continue after adolescence and need to be further investigated into adulthood.

6.4 ASPECTS OF AIRWAY MECHANICS IN RELATION TO PRETERM BIRTH AND EARLY BRONCHIAL OBSTRUCTIVE SYMPTOMS

6.4.1 Compliance of the respiratory system (C_{rs}) after preterm birth

In Study 1, C_{so} was the only measured lung function variable that was associated with BPD severity. It was also associated with respiratory symptoms. Except for infants with moderate/severe BPD and infants reporting symptoms at 6 months, measured values were within normal range and increased with age. Low compliance of the respiratory system in the neonatal period after preterm birth has been shown to be associated with BPD severity in other studies.(81, 107) Improvements during infancy and normalization compared with the control group are shown in those studies. Hjalmarson et al. and Schmalisch et al. found lower values in BPD, but after normalization for lung volume the significance disappeared. However, both studies used FRC_{He}, which has been shown to be lower in BPD than in non-BPD. In our study, we used FRC_{pleth}, which also includes trapped gas, making this comparison difficult. We compared with published reference values that take gestational age and body length into account.(69) For completeness, we also normalized values for weight, which did not change the conclusion (not published). A relationship between early measured C_{so} and wheezing illness and asthma during childhood has been shown in studies after term birth.(108, 109) The underlying pathophysiological mechanism for reduced C_{so} is complex,

involving the total respiratory system and is affected by alveoli as well as bronchi and chest wall. A possible explanation for why early measured C_{so} is associated with both BPD severity and respiratory symptoms is that hyperinflation, which stiffens the lungs, and the level of alveolarization, both lower respiratory compliance.

In summary, compliance of the respiratory system measured in both sedated and un-sedated infants early in life, has been shown to be related to later lung function and respiratory symptoms and is therefore suggested to be tested as a marker of pulmonary outcome after preterm birth.

6.4.2 Impulse oscillometry after to preterm birth and in relation to asthma phenotypes

In Study 2, airway mechanics measured using the IOS technique showed higher values for all indices (R_5, R_{5-20}, AX) among preterm children as compared with controls. IOS variables were not different between the two preterm groups (gestational weeks 22 – 24 and 25 – 26). In follow-up studies of children, with and without BPD, IOS results distinguished between preterms and term controls as well as between preterm groups with and without BPD.(110) (93) Both studies report some concordance for IOS variables and FEV_1, however not for the same indices. In our study, almost all participants had either moderate or severe BPD, making comparison difficult. We can only speculate into the reasons for a lack of association between the extreme preterm groups and IOS indices, in despite of significant spirometric differences. Perhaps IOS has lower sensitivity to discriminate the affected lung region in the most preterm individuals from the overall effect on airways seen after preterm birth. Or, since IOS is performed during tidal breathing without effort, airway closure induced by deep breaths or forced expirations, as during spirometry, will not interfere with the measurement.

In Study 3, IOS results at age 16 showed significantly higher estimated medians for R_5, R_{5-20}, and $AX^{0.5}$ in moderate-to-late preterm males compared with term males. Although a similar trend was seen for females, a significant difference was only seen for R_5, despite significant spirometric differences also between moderate-to-late preterm and term females. To our knowledge, this is the first study using IOS in follow-ups after moderate-to-late preterm birth.

In Study 4, the indices suggested to mirror peripheral airways R_{5-20} had a higher estimated mean in both active asthma groups compared with the never asthma group. It was also shown that the heterogeneity of resistance measured over the airway system was not different

between never asthma and early transient asthma. However, both active asthma groups, particularly the early persistent group, showed skewed distributions with very high values in the 90th percentile. The notion that respiratory mechanics measured using IOS could give additive information compared with spirometric values in active asthma has been shown in studies addressing asthma treatment, asthma control and severity also including bronchial hyper-reactivity without respiratory symptoms, thus supporting our findings.(111, 112)

In summary, Study 2, Study 3 and Study 4 show that for the active asthma phenotypes and the asthma-like syndrome seen after preterm birth, all being bronchial obstructive entities associated with increased smooth muscle airway tone and bronchial hyper-reactivity, the IOS technique has the potential to identify reactive airways. Frequency dependence of resistance (fdR, an index of bronchial obstruction) and AX (sensitive to peripheral bronchial obstruction) both showed increased values after preterm birth and in active asthma phenotypes groups, but not early transient asthma, despite reduced lung function in all groups studied. Hence, the mechanism for bronchial obstruction is different in the early transient asthma phenotype group compared to both active asthma and in the bronchial obstructive disease seen after preterm birth.
Indeed, since the success rate of IOS measurements was also higher than conventional spirometry, primarily because this technique only requires passive cooperation from investigated subjects, it makes IOS a feasible technique particularly in children too young to perform spirometry.

6.5 CLINICAL SYMPTOMS IN RELATION TO LUNG FUNCTION AFTER PRETERM BIRTH

In Study 1, a third of infants reported respiratory symptoms at 6 months and the proportion increased up to almost half at 18 months of age. In contrast to the modest association between BPD severity and lung function, we found a clear association between reported respiratory symptoms and reduced lung function. Both lung mechanics (C_{so}) and lung function measured with TVRTC and RVRTC techniques showed significantly lower values among those reporting symptoms at both 6 and 18 months of age. Associations between respiratory symptoms and lung function after very and extreme preterm birth have only been investigated in a few studies during early childhood. Fakhoury et al. that showed lower V_{max}FRC at 6 (but not at 12 and 24) months of age among those who reported cough but not wheeze, and

Robin et al. found that recurrent wheezing was not associated with reduced lung function, except increased RV among the extreme preterm children.(75, 91)

In Study 2, 40 % of children born preterm, compared with 15 % of controls, reported respiratory wheeze or use of asthma medication (asthma-like disease) in the last 12 months before follow-up at 6½ years. Within the preterm group, there was an association of asthma-like disease to lower lung function measured using spirometry (lower z-scores for FEV_1 and FVC) and altered airway mechanics (higher AX). The EPIcure study reported that 25 % of extreme preterm children at 11 years of age had a current diagnosis of asthma.(84) They also studied the association between lung function and respiratory morbidity (wheeze ever) using spirometry, inert-gas washout and plethymography. They found associations to FEV_1, FEV_1/FVC ratio and specific resistance, but not to LCI or RV/TLC. The latter three are thought to represent more peripheral airways.(33)

In Study 3, females born moderate-to-late preterm reported more respiratory symptoms at both 8 and 16 years of age than females born at term, in spite of less reduced lung function compared with term females than preterm males with term males. Respiratory symptoms were, as expected, negatively associated with lung function in the whole cohort but not within the moderate-to-late preterm group. Gender differences in respiratory health at 19 years of age have been reported after preterm birth and are well recognized in the general term population.(113, 114) It is suggested that this could be explained by different patterns of lung growth between genders. Males have smaller airways in relation to lung size before puberty and this change in puberty makes males less prone than females to respiratory symptoms as adults. However, moderate-to-late preterm females reported more respiratory symptoms already before puberty in our study, thus making this explanation less probable in the group of moderate-to-late preterm children.

In Study 1 and Study 2, respiratory morbidity such as wheeze and asthma-like symptoms during infancy and childhood were associated with lung function decrements seen after very and extreme preterm birth. This was not seen after moderate-to-late preterm birth in late adolescence. The full nature of altered airways and lung parenchyma is not completely understood and needs to be further investigated. However, lung function tests in follow-up after very and extreme preterm birth could be suggested to identify individuals at risk of respiratory morbidity and possible treatment.

6.6 LIMITATIONS AND STRENGTHS

The major strength of this thesis is that the included studies are based on data collected prospectively.

Study 1 and Study 2 included the vast majority of infants with BPD or the most extremely preterm children and there was low loss to follow-up, both facts reducing the risk of selection bias. In the two studies based on the BAMSE cohort, the size and limited loss to follow-up minimizes random errors. In those two studies, a selection bias could have occurred at recruitment (75 % of eligible children included) and at follow-up. To evaluate possible selection bias at follow-up, study populations in Study 3 and Study 4 were compared with the original BAMSE cohort. With respect to population characteristics, the group of children who provided lung function data in Study 3 did not differ to any major extent from the original cohort. In Study 4, individuals who participated in the clinical testing were more often female, had more parental atopy and less maternal smoking during pregnancy than those who did not participate, which might to some extent limit the generalizability.

Gestational age was obtained from the Swedish Medical Birth Registry and in more than 95 % of the pregnancies gestation was determined using ultrasound. The narrow border between gestational age groups could introduce a risk of non-differential misclassification, however that would likely result in an underestimation of the true differences in lung function between the groups.

A limitation and a possible random error of Study 1 is that there are different numbers of missing values for the measured variables, due to insufficient quality of measurement and preterm awaking (despite sedation). It was unlikely that this differed between groups and therefore we believe results were not affected. However, this reflects the challenges to perform lung function tests in sedated infants. In Study 2, the success rate for spirometry was low, but anticipated because of the young age at follow-up, which could introduce a selection bias if children with more severe respiratory morbidity where less successful in performing lung function tests. If so, we could have underestimated the differences between groups.

A major limitation of Study 1 is the absence of a control group, which for ethical reasons was difficult to access.

Misinterpretation due to inappropriate reference data cannot be excluded in Study 1.

A limitation of Studies 2 – 4 is that at early school age, mean spirometric z-scores were not centered at zero. Control groups had approximately 0.5 higher z-scores for spirometric variables at 8 years than the published reference population.(25) This could introduce an underestimation of the frequency of low lung function in our preterm groups in that age span. The effect is likely to be non-differential and not affect comparisons between groups.

One of the major limitations in all but Study 2 is that examination after bronchodilation was not performed. Post-bronchodilator measurements would have been desirable in order to explore whether the observed impairments are due to static changes after remodeling of the airways, impaired airway growth or reversible airway obstruction.

Finally, recall bias could have influenced the classification into symptom groups and prevalence of asthma and asthma-like disease due to the use of questionnaires at the different time points at follow-up.

7 ETHICAL CONSIDERATIONS

In Study 1, all parents were informed of the purpose of the procedures and informed consent was obtained for all measurements. To perform ILFT, the child has to be sedated by orally administrated choral hydrate, which has a bitter taste and could lead to some discomfort for the child. The sedation method is recommended by consensus publications for ILFT. During the time period of recruitment the infant lung function procedures used in the study were implemented as standard follow-up of children with BPD at our center.
In Studies 2 – 4, lung function tests were performed in cooperation with the child/adolescent, who was awake. Lung function tests do not involve large efforts or painful procedures. The lung function methods used are well standardized and common in normal clinical setting worldwide.

8 CONCLUSIONS

- The majority of very and extreme preterm born children have expiratory flow values below the normal range at infancy. At early school age, this is less pronounced but still indicates a significantly reduced lung function. Lung function in adolescence after moderate-to-late preterm birth is lower than after term birth.
- Children born very and extreme preterm have impaired lung function compared with published reference values and term controls, but early staging of BPD severity is insufficent as a prognostic marker of lung function.
- Early measured lung function after preterm birth is associated with respiratory symptoms during infancy and childhood, and therefore suggested to identify infants at risk of respiratory mobidity.
- No catch-up of lung function was seen in the first two years of life after very and extreme preterm birth, or from 8 to 16 years after moderate-to-late preterm birth. This was also observed in early childhood asthma phenotypes between 8 and 16 years of age.
- Childhood asthma phenotypes including early transient disease, based on onset and duration of symptoms, were all negatively associated with FEV_1 at 16 years of age.
- The bronchial obstructive pattern, measured by spirometry and IOS, seen in adoloescense in the group with early transient asthma is different than the obstructive patterns seen in active asthma phenotype groups and after preterm birth. However, the bronchial obstructive pattern in the active asthma phenotypes at 16 years and after preterm birth at 6 and 16 years of age show similarities.
- IOS, which is suitable for lung function measurement in children too young to perform spirometry, may identify children with low lung function after preterm birth and those with active asthma.

9 CLINICAL REMARKS

- The absence of catch-up growth of lung function after preterm birth or in early asthma phenotypes indicates that low initial lung function after preterm and term birth is associated with tracking of reduced lung function during childhood.
- Children born moderate-to-late preterm are at risk of reaching adulthood with a modest, but concerning, reduction of lung function.
- Both time of onset and persistance of asthma symptoms are associated with lung function impairment.
- The combination of data from spirometry and IOS provides new insights about asthma phenotypes and indicates similarities between active asthma and asthma-like disease after preterm birth, which need to be further investigated.
- Preterm birth interrupts normal lung development, which affects lung function later in childhood. Measurements of lung function may identify children at risk for respiratory morbidity and provide insights into long-term sequel of preterm birth.
- Regular assessments of lung function from infancy, during childhood and possibly through life, is therefore suggested to be an important tool when monitoring individuals born preterm, including moderate-to-late preterm birth.
- Intervention studies on treatment of asthma-like symptoms after preterm birth are highly warranted and the understanding of lung function in infancy and during growth could improve the basis for such research.

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10 SVENSK SAMMANFATTNING

Bakgrund: Lungornas utveckling påverkas under fostertiden och barndomen av tidpunkten, längden, typen och intensiteten av diverse händelser, sjukdomsprocesser och omgivningsfaktorer. En vanlig sådan händelse är förtidig födsel. Ungefär 11 % av alla graviditeter är kortare än 37 fullgångna graviditetsveckor, vilket är definitionen på förtidig eller prematur födsel. Några få procent av alla födslar är mycket förtidiga (tidigare än 32 veckor). Incidensen för den mest extremt förtidigt födda gruppen, tidigare än 27 veckor, var 3/1 000 födda i den svenska multicenterstudien EXPRESS (Extremely Preterm Infants in Sweden Study).

Bronkopulmonell dysplasi, eller också benämnt kronisk lungsjukdom efter förtidig födsel, är det kanske vanligaste och allvarligaste kroniska resttillståndet efter extrem förtidig födsel. I EXPRESS-studien hade nära alla deltagare diagnostiserats neonatalt med någon grad av sjukdomen, varav 73 % med måttlig eller svår BPD. Uppföljningar från spädbarnsåldern till ung vuxen ålder har visat kvarstående luftvägssjuklighet och nedsatt lungfunktion. Den kliniska bilden av luftvägssjukdomen efter spädbarnstiden uppvisar en astmalik bild inkluderande återkommande pip och väs i luftrören, ökad bronkiell hyperreaktivitet samt obstruktiv lungfunktionsnedsättning. Man bör dock inte använda begreppet astma för detta tillstånd eftersom astma i allmänhet definieras som en inflammatorisk luftvägssjukdom. Lungsjukdomen efter förtidig födsel är emellertid inte associerad med luftvägsinflammation. Astma och astmalika tillstånd är mycket vanliga under barndomen också efter födsel i fullgången tid.

Över 30 % av alla barn i befolkningen har haft enstaka eller återkommande episoder av astmalika symtom, främst vid luftvägsinfektioner, under sina första levnadsår. För majoriteten är prognosen god och mindre än en tredjedel har kvar problemen efter sex års ålder. I syfte att beskriva förlopp av astmasjukdom, välja och utveckla behandlings- och preventionsstrategier, har flera astmafenotyper beskrivits. Dessa fenotyper är oftast retrospektivt beskrivna och har alltså föga värde för klinkern som står med ett barn med astmalika symtom framför sig. Att identifiera kliniska astmafenotyper med tillräckligt god sensitivitet och specificitet i klinisk vardag för att avgöra prognos och välja behandling, har visat sig vara svårt, men sannolikt kan information om lungfunktion, genetik och omgivningsfaktorer vara en väg framåt. Den vanligaste fenotypen av småbarnsastma är den med tidig debut och oftast symtomfrihet efter 4 – 5 års ålder; s.k. transient early

asthma/wheeze (ung. ”tidig övergående astma”) som vi på svenska ofta kallar ”övergående förkylningsastma”. Övergående förkylningsastma har visats ge låg lungfunktion redan under spädbarnstiden och kvarstående lägre lungfunktion som ung vuxen, trots att luftvägssymtomen upphör redan under småbarnstiden. Huruvida det astmalika tillståndet som ses efter förtidig födsel ska betraktas om en unik astmafenotyp kan ifrågasättas. Likheten mellan övergående småbarnsastma och tillståndet efter förtidig födsel (tidigt nedsatt lungfunktion, ej typisk allergisk inflammation) kan göra jämförelser intressanta.

Huvudfrågeställningar

- Hur påverkas lungfunktionen från spädbarnstiden till adolescensen i relation till förtidig födsel och tidigt utvecklad astma i barndomen?
- Kan likheter och skillnader i lungfunktion hos förtidigt födda individer och personer med olika astmafenotyper i barndomen ge information om mekanismerna bakom luftvägsobstruktionen hos dessa tillstånd?

Delstudie 1: Lungfunktion vid 6 och 18 månader efter förtidig födsel i relation till svårighetsgrad av bronkopulmonell dysplasi (BPD)

Femtiofem extremt förtidigt födda barn med olika grader av BPD undersöktes med flera olika typer av lungfunktionsmetoder under inducerad sömn vid 6 och 18 månader efter födseln. Majoriten av barnen hade vid både undersökningstillfällena definitionsmässigt nedsatta expiratoriska utandningsflöden (< -1.96 standardavvikelser). Utom lägre compliance av respiratoriska systemet (ung. elastiska egenskaper), som var lägre hos barn med måttlig/svår BPD än hos lindrig BPD, fann vi inga skillnader i lungfunktion mellan svårighetsgraderna. Emellertid fann vi statistiskt signifikant sämre lungfunktion i flertalet av de uppmätta lungfunktionsmått hos de barn för vilka luftvägssymtom rapporterades vid 6 och/eller 18 månader, jämfört med hos dem som var symtomfria.

Slutsatsen av undersökningen var att extremt förtidigt födda barn hade låg lungfunktion vid 6 och 18 månader utan klar relation till BPD-grad och att luftvägssymtom var tydligt associerade med sämre lungfunktion.

Delstudie 2: Lungfunktion hos 6 år gamla barn som föts extremt tidigt - en populationsbaserad kohortstudie (EXPRESS)

Drygt hälften av deltagarna i den nationella svenska multicenterstudien EXPRESS, som inkluderat alla extremt förtidigt födda barn i Sverige (< 27 födelsevecka) inbjöds vid 6 års ålder till studien. Vid uppföljningen gjordes lungfunktion med två metoder (spirometri och impulsoscillometri). Lungfunktionsdata från 153 förtidigt födda barn och 157 kontrollbarn, födda i fullgången tid, kunde analyseras.
Bägge metoderna visade statistiskt signifikant nedsatt lungfunktion för den prematurfödda gruppen. Undergruppen av barn födda under födelsevecka 22 till och med 24 påvisades ha sämst lungfunktion. Nära hälften av dessa hade ett forcerat expiratoriskt flöde vid 1 sekund (FEV_1) under den statistiska femte percentilen (förväntat 5 %) och en fjärdedel hade en maximal utandad volym (FVC) under samma percentil. Signifikant förbättring av lungfunktionen sågs hos 28 % av de prematurfödda efter inandning av luftrörsvidgande läkemedel jämfört med 10 % av konrollerna. Om barnet var fött med låg födelsevikt (*small for gestational age*, SGA) eller med svår BPD gav det bara marginellt sämre lungfunktion jämfört med vid normal födelsevikt respektive lägre allvarlighetsgrad av BPD.

Slutsatserna var att ett stort antal förtidigt födda barn har kliniskt relevant nedsatt lungfunktion vid 6 års ålder, att resultaten visade att luftvägsbehandling bör övervägas och att fortsatta uppföljningar av lungfunktionen bör ske under uppväxten.

Delstudie 3: Lungfunktion vid 8 och 16 års ålder efter måttligt förtidig födsel: En prospektiv kohortstudie.
Vi använde data från BAMSE-kohorten, som ursprungligen hade 4 089 deltagare med prevalensen 4.8 % för gruppen måttligt till lindrigt förtidigt födda (födelsevecka 32 – 36). Lungfunktion med spirometriundersökning vid 8 och 16 år samt impulsoscillometri (IOS) vid 16 års ålder användes vid analyserna. Totalt bidrog 2 621 deltagare, varav 149 prematurfödda och 2 472 fullgångna (födelsevecka 37 – 41) med lungfunktionsdata.
Vid 16 års ålder hade båda könen statistiskt lägre lungfunktion mätt med spirometri (FEV_1 3,4 % lägre för flickor och 4 % lägre för pojkar). Andelen pojkar med lågt FEV_1 (under femte percentilen) var vid 16 års ålder 15 % (kontroller 3,7 %). IOS-resultaten bekräftade lägre lungfunktion för pojkar, men för flickor sågs en trend som inte nådde statistisk signifikans. Longitudinell lungfunktion från 8 till 16 år visade att de prematurfödda barnen inte ökade sin lungfunktion (FEV_1, MEF_{50}, FEV_1/FVC) jämfört med dem som fötts i fullgången tid. Skillnaden kvarstod för flickor, medan skillnaden ökade för pojkar.

Slutsatsen var att både flickor och pojkar födda bara några veckor före beräknad fullgången tid även i tonåren hade lägre lungfunktion, 3,4 % respektive 4 %, jämfört med dem som fötts i fullgången tid. Vidare sågs mellan 8 och 16 års ålder ingen relativ förbättring av lungfunktionen för de förtidigt födda.

Delstudie 4: Astmafenotyper och lungfunktion upp till 16 års ålder – BAMSE-kohorten
Liksom i delstudie 3 användes data från BAMSE-kohorten och lungfunktionsresultaten från 8 och 16 års ålder. Baserat på enkätsvar (1-, 2-, 4-, 8- och 16-års-uppföljningarna) kategoriserades deltagarna i astmafenotyperna: ”aldrig astma” (82 %), ”tidig övergående astma” (6 %), ”tidig kronisk astma” (3 %) samt ”sent debuterande astma” (3 %).
Vid 8-års-uppföljningen bidrog 1 832 deltagare med spirometriresultat och vid 16-års-uppföljningen bidrog 2 056 med spirometriresultat och 2 453 med IOS-resultat. Spirometriresultaten visade att alla tre astmagrupperna hade statistiskt lägre lungfunktion vid 16 års ålder jämfört med individer utan astma. IOS-resultaten vid 16 års ålder visade att båda grupperna med aktiv astmafenotyp hade statistiskt högre värden för R_{5-20} och AX (”index” för perifer luftvägsobstruktivet) medan dessa värden inte skiljde sig mellan fenotypen ”tidig övergående astma” och gruppen ”aldrig astma”. Vidare sågs ingen relativ förbättring av lungfunktion mellan 8 och 16 år för någon av astmafenotyperna. I stället sågs en ökning av skillnaderna för FEV_1 för de aktiva astmafenotyperna jämfört med fenotypen som aldrig haft astma.

Slutsatsen blev att oavsett astmafenotyp hade individer med astma någon gång under barndomen lägre spirometriskt mätt lungfunktion vid 16 års ålder. Ingen relativ förbättring av lungfunktionen mellan 8 och 16 år sågs jämfört med barn som aldrig haft astma. IOS-resultaten visade att grupperna som hade astmafenotyper med aktiv astma var associerade med fynd som kan förklaras av obstruktivitet i små luftvägar.

Konklusioner

- Majoriteten av extremt förtidigt födda barn hade under spädbarnsperioden maximala expiratoriska lungfunktionsvärden som var under nedre gränsen för normalvärden. I tidig skolålder var detta mindre uttalat, men en påtaglig reduktion av lungfunktionen kvarstod. Lungfunktionen var, vid 16 års ålder, lägre för dem som var födda bara några veckor förtidigt jämfört med dem som fötts i fullgången tid.

- Tidig gradering av svårighetsgrad för BPD gav begränsad information om senare lungfunktion och är en otillräcklig markör för lungfunktionsutveckling under barndomen.
- Tidigt mätt lungfunktion efter förtidig födsel var associerad med luftvägssymtom och kan därför identifiera individer med risk för luftvägssjukdom.
- Alla undersökta astmafenotyperna hade vid 16 års ålder lägre lungfunktion än gruppen som aldrig haft astma.
- Longitudinellt såg man ingen relativ förbättring av lungfunktionen under uppföljningsperioderna efter förtidig födsel eller efter tidig astmadebut.
- IOS-metoden, som är enkel att använda på yngre barn som ännu inte kan genomföra spirometri, identifierar låg lungfunktion efter tidig födsel och individer som har astmafenotyper med aktiv astmasjukdom.

Klinisk slutsats

- Lungfunktionsmätning kan identifiera barn med risk för symtom från luftvägarna och kan öka kunskapen om framtida lungutveckling. Jag föreslår därför att förtidigt födda barn ska genomföra undersökningar av lungfunktionen tidigt och regelbundet, upp till vuxen ålder.

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