Alveolar function following extremely preterm birth

Emma Satrell

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2022

UNIVERSITY OF BERGEN

Alveolar function following extremely preterm birth

Emma Satrell

Thesis for the degree of Philosophiae Doctor (PhD)
at the University of Bergen

Date of defense: 16.12.2022

Year: 2022

Title: Alveolar function following extremely preterm birth

Name: Emma Satrell

Print: Skipnes Kommunikasjon / University of Bergen

Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

- Marie Curie

Contents

Scientific environment

The scientific work leading to this PhD project was started as part of the Medical Student Research Program, University of Bergen. The further work emerged from the PhD program at the Department of Clinical Science, Faculty of Medicine, University of Bergen, Norway. The research was conducted within the frames of the "WestPaed Research Group".

Professor Trond Markestad and Professor Thomas Halvorsen supervised me during the Medical Student Research Program. Dr Maria Vollsæter has been my main supervisor, while Professor Thomas Halvorsen and Professor Einar Thorsen have been my co-supervisors during the PhD program. Ola Røksund has contributed with important supervision when it comes to learning and understanding the different pulmonary function tests.

The thesis is based on two population-based cohorts of children born extremely preterm in the 1980's and 1990's respectively, and a cohort of children born following previable preterm premature rupture of membranes in the beginning of the 21st century. The extremely preterm cohorts were established in 2001 by Professor Thomas Halvorsen.

Acknowledgements

There are so many people who deserve thanks for their contribution to this thesis. First, the children, adolescents and adults who participated in the studies and their parents made this work possible and deserve a huge thanks. As a clinician, the most rewarding part for me is all the encounters with people, and to meet the participants in the studies was no exception.

As a first-year medical student at the University of Bergen, I first met Thomas Halvorsen at a meeting arranged by the faculty where there was given information on the Medical Student Research Program ("Forskerlinjen") in 2009. He and Ola Røksund enthusiastically introduced me to the pulmonary laboratory at the "old" children's clinic at Haukeland University Hospital and quickly made me feel "at home" and included in the group, even though I at that time was quite a novice when it comes to pulmonary testing and paediatrics. They introduced me to the world of research that has been more fun, exciting, and rewarding than I could ever have imagined. Over the years since 2009, I have had the opportunity to meet, collaborate, and learn from so many talented people, and I am forever grateful for that.

I have had the pleasure of having Thomas Halvorsen as my supervisor both during the Medical Student Research Program and during the PhD project. Getting to know him has been a true privilege, and it is his merit that I have had the opportunity to do this work. Even though I have periodically been absent from the research group when moving to Oslo and starting my clinical career as a medical doctor, he has shown great patience with me, and I have always felt highly prioritized and appreciated. He has given me so many opportunities and I will always be grateful that it was him that supervised me through all these years- I could never have wished for a better supervisor!

Maria Vollsæter, my main supervisor during this PhD project, has been a highly valued person in my life during the past years. She has become not only my supervisor, but also an important motivator and friend. Her extensive knowledge and experience have been truly valuable, and her pragmatic and structured way of working has taught med so much. My appreciation to Maria is enormous.

My co-supervisor Einar Thorsen has provided such valuable knowledge regarding pulmonary physiology. Moreover, his patience and calm have added an appreciated complement to the other supervisors.
Ola Røksund has contributed with invaluable knowledge regarding pulmonary testing and lung function. His dedication and enthusiasm have undoubtedly been important for my development during these years, and for the results. Moreover, I am very grateful to him for always making me and other young and unexperienced students and colleagues feel like a natural, and valued part of the research environment.
I would like to thank my employer, the Department of Paediatric and Adolescent Medicine at Vestre Viken, and especially Anders Morken for supporting me during the last years while completing the PhD work. Anders has shown great flexibility and encouraged me in creating the path forming “my own paediatric career”. I am greatly thankful for this opportunity.
Huge thanks also to Hege Clemm for valuable help during the work with this project, and to Mariann Bentsen, Hallvard Reigstad, Ansgar Berg and Gottfried Greve for the collaboration regarding the PPROM study. Geir Egil Eide and Karl Ove Hufthammer have contributed with valuable help to the statistics.
At the end I would like to thank my friends, family, and colleagues for all your support, and my parents Ylva and Per-Olof for always believing in me no matter what.
Lastly, Erling- my most significant other for your unconditional love and for being my biggest fan. You never stop believing in me and give me endless support regarding all my projects. You and our son Alfred, who was born while I was completing this work, are the biggest joy of my life. I am more grateful than I can put into words.

Emma Satrell, August 2022.

Summary of thesis

Background: Extremely preterm (EP) birth occurs before normal lung development is complete and disturbs acinar growth and differentiation. Reduced surface area and thicker alveolar membranes cause gas exchange impairments that may persist throughout childhood and adulthood. Premature rupture of membranes (PPROM) may further affect this development. Measuring the diffusion capacity of the lungs (DL_{CO}) can be used as an indirect measure of impaired alveolar-capillary function.

Aims: To investigate the development of lung diffusing capacity from mid childhood to early adulthood in individuals who survived EP birth and in subjects who survived PV-PPROM (PPROM before fetal viability is possible outside the uterus), with additional comprehensive cardiopulmonary investigations in the latter group.

Methods: Two area-based cohorts born at ≤28 weeks' gestational age (GA) or with birthweight ≤1000 g in 1991–2 (n = 35) and 1982–5 (n = 48) were assessed by lung diffusing capacity measurements at ages 10 and 18 years and 18 and 25 years, respectively, together with individually matched term-born controls. In addition, a group of children (n = 11) born after PV-PPROM, with individually matched controls born at the same GA without PPROM, were assessed by cardiopulmonary exercise testing, lung function testing, and echocardiography at 10 years of age.

Results: The diffusion capacity of the lungs, and in particular the membrane component, was parallelly shifted to reduced levels in individuals born EP from mid-childhood to young adulthood, compared with matched term-born controls. There were neither signs of catch-up growth, nor signs of early-onset reduction of diffusion capacity at 25 years of age. Compared with preterm individuals without PPROM, the PV-PPROM group had lower lung function, reduced maximal oxygen uptake and signs of mild pulmonary hypertension on echocardiography.

Conclusion: Impaired gas exchange in the lungs after EP birth persisted from mid-childhood to young adulthood. During the study period, there were no signs of catch-up growth or signs of early-onset reduction of diffusion capacity. People born after PV-PPROM are a vulnerable subgroup of EP-born individuals.

Sammenfatning av avhandling

Bakgrunn: Ekstremt prematur (EP) fødsel skjer før normal lungeutvikling er fullført, og forstyrrer vekst og differensiering av alveolene. Mindre areal og tykkere membraner i alveolene gjør at lungenes evne til å drive gassutvikling blir redusert, og dette kan vedvare gjennom barndommen og voksenlivet. Utviklingen kan ytterligere påvirkes av prematur vannavgang (PPROM). Måling av lungenes diffusjonskapasitet (DL_{CO}) kan brukes som et indirekte mål for nedsatt alveolær-kapillær funksjon.

Mål: Undersøke longitudinell utvikling av lungenes diffusjonskapasitet hos individer født EP og hos personer som overlevde PV-PPROM (PPROM før føtal levedyktighet er mulig utenfor livmoren), med ytterligere omfattende kardiopulmonale undersøkelser i sistnevnte gruppe.

Metode: To områdebaserte kohorter født ved gestasjonsalder (GA) ≤28 uker eller med fødselsvekt ≤1000 g i 1991–2 (n = 35) og 1982–5 (n = 48) og individuelt matchede terminfødte kontroller utførte måling av diffusjonskapasitet ved alder henholdsvis 10 og 18 år og 18 og 25 år. I tillegg ble en gruppe barn (n = 11) født etter PV-PPROM, samt individuelt matchede kontroller født ved samme GA uten PPROM, undersøkt med fysisk belastningstest, lungefunksjonstesting og ekkokardiografi ved 10 års alder.

Resultat: Lungenes diffusjonskapasitet, og spesielt membrankomponenten, var parallelt forskjøvet til nedsatte nivåer hos individer født EP fra midten av barndommen til ung voksen alder, sammenlignet med matchede terminfødte kontroller. Det var ingen tegn innhentingsvekst, eller tegn til tidlig begynnende reduksjon av diffusjonskapasitet ved 25 års alder. Sammenlignet med prematurfødte individer uten PPROM, hadde PV-PPROM-gruppen dårligere lungefunksjon og redusert maksimalt oksygenopptak, og viste tegn på mild pulmonal hypertensjon ved ekkokardiografi.

Konklusjon: Nedsatt gassutveksling i lungene etter EP fødsel vedvarte fra midten av barndommen til ung voksen alder. Det var under studieperioden ingen tegn til innhentingsvekst eller tidlig begynnende reduksjon av diffusjonskapasitet. Personer født etter PV-PPROM er en sårbar undergruppe av EP-fødte individer.

List of publications

Paper I

Satrell E, Roksund O, Thorsen E, Halvorsen T. Pulmonary gas transfer in children and adolescents born extremely preterm. *Eur Respir J*. 2013;**42**(6):1536–44.

Paper II

Bentsen MH, Satrell E, Reigstad H, *et al*. Mid-childhood outcomes after pre-viable preterm premature rupture of membranes. *J Perinatol*. 2017;**37**(9):1053–9.

Paper III

Satrell E, Clemm H, Roksund O, *et al*. Development of lung diffusion to adulthood following extremely preterm birth. *Eur Respir J*. 2022;**59**(5):2004103.

Abbreviations

AGA	appropriate for gestational age
AMA	American Medical Association
ATS	American Thoracic Society
BPD	bronchopulmonary dysplasia
BW	birthweight
CH_4	methane
C_2H_2	acetylene
CHQ	Child Health Questionnaire
CI	confidence interval
CLD	chronic lung disease of infancy
CO	carbon monoxide
CO_2	carbon dioxide
COHb	carboxyhaemoglobin
COPD	chronic obstructive pulmonary disease
CPAP	continuous positive airway pressure
CV	coefficient of variation
DL_{CO}	diffusing capacity of the lung for carbon monoxide
DL_{NO}	diffusing capacity of the lung for nitric oxide
D_M	membrane conductivity (or membrane factor)
EP	extremely preterm
ERA	endothelin receptor antagonist
ERS	European Respiratory Society
ERV	expiratory reserve volume
FEF	forced expiratory flow
$FEF_{25–75}$	forced expiratory flow at 25%, 50%, and 75% of forced vital capacity
FEV_1	forced expiratory volume in 1 second
FiO_2	fraction of inspired oxygen
FRC	functional residual capacity
FVC	forced vital capacity

GA	gestational age
GLI	Global Lung Function Initiative
GOLD	Global Initiative for Chronic Obstructive Lung Disease
Hb	haemoglobin
HRCT	high-resolution computed tomography
HUS	Haukeland University Hospital
IB	intra-breath (technique)
IgE	immunoglobulin E
iNO	inhaled nitric oxide
IPF	idiopathic pulmonary fibrosis
IPPV	intermittent positive pressure ventilation
IRV	inspiratory reserve volume
ISAAC	International Study of Asthma and Allergies in Childhood
IUGR	intrauterine growth restriction
IVH	intraventricular haemorrhage
K_{CO}	transfer coefficient of the lung for carbon monoxide
LGA	large for gestational age
LISA	less invasive surfactant administration
LMP	last menstrual period
MLM	mixed linear model
MRI	magnetic resonance imaging
n-CPAP	nasal continuous positive airway pressure
NEC	necrotizing enterocolitis
NICHD	National Institute of Child Health and Human Development
NICU	neonatal intensive care unit
NIPPV	nasal intermittent positive pressure ventilation
NO	nitric oxide
O_2	oxygen
OR	odds ratio
PDA	patent ductus arteriosus
PDE-5	phosphodiesterase type 5

PEF	peak expiratory flow
PH	pulmonary hypertension
PIH	periventricular–intraventricular haemorrhage
PMA	post-menstrual age
pO_2	partial pressure of oxygen
PPHN	persistent pulmonary hypertension in the newborn
PPROM	preterm premature rupture of membranes
PROM	premature rupture of membranes
PVD	pulmonary vascular disease
PVL	periventricular leukomalacia
PV-PPROM	previable preterm premature rupture of membranes
PVR	pulmonary vascular resistance
PVRI	pulmonary vascular resistance index
Raw	airway resistance against tidal respiration
RDS	respiratory distress syndrome
ROP	retinopathy of prematurity
ROS	reactive oxygen species
RV	right ventricle; residual volume
SB	single-breath (technique)
SD	standard deviation
SGA	small for gestational age
SPT	skin prick test
TAPSE	tricuspid annular plane systolic excursion
TLC	total lung capacity
TL_{CO}	transfer factor for carbon dioxide
TR	tricuspid regurgitation
TRJV	tricuspid regurgitation jet velocity
VA	alveolar volume
V_C	volume of alveolar–capillary blood
VC	vital capacity
VD	dead space volume

VI	inspiratory volume
VLBW	very low birthweight (<1500 g)
VO_2	oxygen uptake
VTG	thoracic gas volume
WSPH	World Symposium on Pulmonary Hypertension
WU	Wood unit

1 General introduction

1.1 Preterm birth

Preterm birth is defined as birth occurring before 37 completed weeks of gestation, or fewer than 259 days since the first day of the mother's last menstrual period (LMP).[1] Preterm birth is categorized into subgroups, based on gestational age (GA):

- Extremely preterm (EP): <28 weeks.
- Very preterm: 28 to <32 weeks.
- Moderate to late preterm: 32 to <37 weeks.

Infants may be categorized by birthweight (BW) according to the GA. Although a consensus definition is lacking, the most commonly used definition by clinicians is based on percentiles of a distribution of BW for GA derived from a reference population:[2, 3]

- Small for GA (SGA): BW <10th percentile for GA.
- Appropriate for GA (AGA): BW between 10th and 90th percentiles for GA.
- Large for GA (LGA): BW >90th percentile for GA.

It is important to differentiate between the terms SGA and intrauterine growth restriction (IUGR). SGA is a statistical definition, and an SGA fetus may be healthy and 'meant to be small'—in other words, constitutionally small. IUGR, on the other hand, is a clinical definition and refers to a condition whereby the fetus fails to reach its biological growth potential,[4] and consequently is at increased risk of complications and death, compared to AGA infants.[5, 6]

1.1.1 Epidemiology of preterm birth

Worldwide, around 15 million children are born preterm annually, with the majority born after 32 weeks' gestation. The global preterm birth rate was estimated to be 10.6% in 2014, varying from around 5% in some European countries to around 12% in low-income countries (reaching up to 18% in some parts of Africa).[7] There are wide geographical differences in survival rates,[1, 8] and premature birth is the most common cause of death in children aged under 5 years worldwide.[9] The rate of preterm births

in Norway is around 5.9%, with EP birth rate being relatively stable at around 0.4–0.5%.[10, 11] Around 300 children are born EP every year in Norway.

Survival among preterm infants, and especially those born EP, has improved over the past decades, most likely due to advances in perinatal care.[12-14] The 1-year overall survival rate for EP live-born infants admitted to neonatal intensive care units (NICUs) in Scandinavia has been relatively stable at around 70% since the turn of the millennium,[11, 15] with further improved survival from 2004–2007 to 2014–2016 demonstrated by a recent Swedish study.[16]

1.1.2 Causes of preterm birth

Preterm birth is a complex syndrome resulting from multiple pathological processes. The obstetric precursors leading to preterm birth can be divided into three groups: (1) spontaneous labour with intact membranes; (2) preterm premature rupture of membranes (PPROM); and (3) labour induction or Caesarean delivery for maternal or fetal indications.[17]

Several factors, including maternal, obstetric, and fetal, are known to increase the risk of spontaneous premature birth.[18-21] Maternal risk factors include smoking,[22] infections during pregnancy,[23] young and advanced age,[24] and low educational level.[25] Further, recent findings suggest a maternal history of previous preterm delivery could be the strongest maternal risk factor.[19] Obstetric risk factors include multiple gestations,[26] short cervical length,[27] and pre-eclampsia.[19] Fetal risk factors include malformations and fetal infection.[21, 28] However, the majority of preterm births have not been found to be associated with any known risk factors.[19]

1.1.3 Premature preterm rupture of membranes

1.1.3.1 Definitions and epidemiology

Premature rupture of membranes (PROM) is defined as spontaneous rupture of fetal membranes before onset of labour. Rupture of fetal membranes before 37 weeks' gestation is termed PPROM. PROM occurs in around 3% of all pregnancies and in a third of all preterm births.[29] Previable PPROM (PV-PPROM), which occurs typically before 23–24' weeks gestation, i.e. before fetal viability is possible outside the uterus,

complicates approximately 0.4–1% of all pregnancies, depending on the definition of viability.[29, 30] Immediate delivery in cases of PV-PPROM will lead to neonatal death, whereas conservative management may lead to extended latency (time from membrane rupture to delivery) and delivery of a potentially viable infant. Reported overall perinatal survival rates are low, and the best predictors seem to be GA at membrane rupture, time from membrane rupture to delivery, and persistent oligohydramnios.[29, 31] Although international guidelines exist on management of PPROM from 24 weeks' gestation,[32] antenatal counselling of women who develop PV-PPROM on their management options remains difficult. Norwegian obstetric guidelines offer termination of pregnancy as one option, particularly when PPROM and oligohydramnios occur before 20 weeks' gestation.[33] By contrast, general obstetric management of PV-PPROM is 'watchful waiting', with the aim to increase GA at delivery.

1.1.3.2 Pathophysiology

The aetiology of PPROM is multifactorial, although intrauterine infection and inflammation both play an important role, especially at early GA.[34, 35] It is hypothesized that weakening of the amniochorionic membrane occurs because of membrane stretch or degradation of the amniochorionic extracellular matrix. Proposed risk factors include low socio-economic status, previous cervical conization, genetic predisposition, amniocentesis, and prior bleeding or preterm labour.[30] Moreover, behavioural factors, such as smoking, substance abuse, and sexually transmitted diseases, are also associated with PPROM.[36]

1.1.4 Neonatal care of the premature infant

In the 1950s, Virginia Apgar developed a scoring system, known as the Apgar score, for assessing the health status of newborns, based on five criteria: heart rate, respiration, reflexes, muscle tone, and skin colour.[37] In 1960, the term neonatology was coined by Alexander Schaffer.[38] Even though there were some advances within the field of neonatology at the time, it was not until the 1970s with the introduction of mechanical ventilation that modern neonatal intensive care began to develop.[39] As a

result, survival rates of infants with BW <1000 g improved from <10% in the 1960s, when treatment of respiratory problems in newborn infants was limited to supplemental oxygen therapy only, to around 35% during the mid-1970s.[39] Since then, various modes of respiratory support have been introduced successively in NICUs, such as intermittent positive pressure ventilation (IPPV) and high-frequency oscillatory ventilation, as well as different modes of non-invasive respiratory support, including continuous positive airway pressure (CPAP) and nasal high-flow therapy. Effective monitoring of blood oxygen saturation levels was enabled by the introduction of pulse oximetry. Further, recognition of the association of unrestricted oxygen supplementation with retinopathy of prematurity (ROP) and blindness has led to recommendations of more moderate oxygen saturation targets.[40]

Use of antenatal steroids in pregnant women with threatening preterm delivery, to accelerate fetal lung maturation, was first introduced in the early 1970s[41] and became widely used from the late 1970s. Treatment with antenatal steroids is associated with an overall average reduction in perinatal death, respiratory distress syndrome (RDS), and intraventricular haemorrhage (IVH),[42] and thus has contributed significantly to reducing neonatal morbidity and mortality following EP birth.

Surfactant lines the alveolar surface and reduces surface tension, thereby preventing atelectasis at end-expiration. Its role was first described in 1959,[43] and exogenous surfactant replacement therapy was first reported in 1980.[44] It became available for clinical use from the late 1980s and has been widely used in NICUs since the early 1990s.[45]

Other factors contributing to improved care of EP infants include aggressive use of antibiotics,[46] optimal neonatal nutrition,[47, 48] effective management of patent ductus arteriosus (PDA),[49, 50] and improved ventilatory support, cannulation, and peripheral and central access. In parallel with improved respiratory support and medical treatment, there has been increased emphasis on family-centred care that encourages parental involvement in the care of their infant, including skin-to-skin or 'kangaroo mother care'.[51, 52] In recent years, improvement to the NICU environment has also received increased attention, with the creation of single-family rooms[53] and

adjustment of sensory stimuli to the infant such as use of cycled lighting to promote the development of circadian rhythms' and noise restriction.[54]

The gradual development of, and progress in, NICU care over the past decades has meant that children born EP in the 1980s and 1990s and those after the turn of the millennium received slightly different neonatal intensive care treatment. The different birth cohorts of EP-born subjects in the studies presented in this thesis were from different decades and therefore were not given identical treatments in the neonatal period.

1.1.5 Ethical considerations in the NICU setting

Ethical considerations are part of daily practice in the NICU. While a pregnant woman's autonomy is at the heart of decision-making before and in early pregnancy, in terms of preventing, planning, or terminating the pregnancy, more emphasis shifts towards the fetus from mid-pregnancy onwards. With advances in medical technology and care of EP infants, the limit of viability has been pushed towards lower GAs. Guidelines on the GA at which active neonatal resuscitation and care should be given vary, and international consensus is lacking.[55] Management of the most immature babies born around 22–24 weeks' gestation constitutes a 'grey area',[56] with a wide variation in recommendations at national, regional, and, in some cases, even institutional levels.[57] Recommendations for management of infants born at 22–24 weeks' gestation often include parental counselling, with emphasis on parental wishes and on individualized care in the best interests of the infant. There is great uncertainty in terms of outcome, particularly at an individual level, in infants born at 22–24 weeks' gestation.[55] While treatment of infants at a GA of 22 weeks is offered rarely in Norway,[58] a few countries, including Sweden where a national consensus guideline was issued in 2016 advocating for more active management of infants born at 22+0 weeks' gestation,[59] have adopted a more proactive approach regarding management of these infants.[16, 60, 61]

Counselling of parents in cases of PV-PPROM is particularly challenging. Survival rates and the likelihood of severe morbidity among survivors differ greatly across studies, and studies of long-term outcome are mostly lacking. PV-PPROM is a

rare phenomenon, with most NICUs having limited experience in managing such cases—this, in turn, makes counselling even more challenging.

Therefore, there is an imperative need of research regarding long-term consequences of EP births and births following PV-PPROM.

1.2 The respiratory system

The primary function of the respiratory system is to perform effective gas exchange in order to maintain cellular homeostasis. The respiratory system comprises an extensive system of conducting airways and respiratory units. The upper airways include the nose and nasal cavities, sinuses, pharynx, and larynx above the vocal cords, while the lower airways consist of the lower part of the larynx, the trachea, bronchi, and the lungs. An optimal system for gas exchange should have open airways to allow gas transport, the largest possible surface area and a thin diffusion barrier for gas exchange, and a steep gas concentration gradient for diffusion to take place. The main goal of lung development is to generate an organ that satisfies these criteria.

1.2.1 Fetal development of the respiratory system

Development of the human respiratory system takes place over three main periods: embryonic, fetal, and postnatal. The fetal period can be divided into the pseudoglandular, canalicular, and saccular stages. Lung development commences with a primitive lung bud at around 4 weeks' gestation and continues throughout fetal life, childhood, and into young adulthood.[62] It is a highly coordinated and complex process, with its underlying regulatory mechanisms still poorly understood. It has been shown that maternal factors, such as nutrition and exposure to tobacco and alcohol during pregnancy, can impact negatively on lung development.[63]

1.2.1.1 The embryonic period (weeks 0 to 6)

The embryonic stage begins during week 4 of gestation with outgrowth of a lung bud from the ventral wall of the primitive foregut. The lung bud, which will go on to form the trachea at one end, bifurcates at the other end on each side of the oesophagus to eventually form the two main bronchi'. Further branching results in the formation of

lobar and segmental bronchi. At the same time, vasculogenesis occurs, with the sixth pair of the aortic arches developing into a vessel plexus that surrounds the lung tubules. By the end of week 6, the respiratory system is recognizable with an emerging adult pattern, comprising central vascular and airway structures that include lobar and segmental branches.[62]

1.2.1.2 The fetal period: pseudoglandular stage (weeks 6 to 16)

The main airways develop through successive branching, resulting in the formation of pre-acinar airways, which are the non-gas-exchanging area of the lungs. A primitive airway epithelium starts to grow and differentiate, along with the development of mucous glands and connective tissue, while mesenchymal cells begin to form cartilage and smooth muscle cells. By the end of this period, all 20 branching generations of the bronchial tree have been established. The pulmonary vasculature branches out following the airways and forms the complete pre-acinar vascular system that corresponds to that of the adult lung. Cuboidal epithelial cells are formed, which are immature type II alveolar epithelial cells that later will secrete surfactant. At this point, no alveolar formation has taken place, which means gas exchange is not yet possible, and therefore, birth is not compatible with life.[62]

1.2.1.3 The fetal period: canalicular stage (around weeks 16 to 24–26)

This stage is marked by the formation of respiratory bronchioli, alveolar ducts, and primitive alveoli, as well as the development of early pulmonary parenchyma. Continuing vascular development results in the formation of a capillary network around the air spaces. The cuboidal epithelial cells further differentiate into type II pneumocytes, from which type I pneumocytes start differentiating. Type I pneumocytes line the alveolar surface, including the blood–air barrier, thus enabling gas exchange. Surfactant production begins at around week 26.[62, 64]

Surfactant is composed of phospholipids, neutral lipids, and protein. It is produced by type II pneumocytes lining the alveoli and is secreted into the airways mainly during the last trimester. The role of surfactant is to reduce surface tension and thus prevent alveolar collapse.[62, 64]

Premature, and in particular EP, infants are born with an immature surfactant system. In the preterm newborn, the resulting high surface tension in the lungs could cause alveolar collapse, resulting in atelectasis, which further increases the work of breathing. Achieving adequate and sufficiently prolonged lung expansion for efficient gas exchange to occur is highly energy-consuming for the infant to maintain the work of breathing, such that many develop respiratory distress syndrome (RDS). Exogenous surfactant has been standard therapy for EP-born infants since the early 1990s. The methods of surfactant administration into the lungs have evolved over the years, with less invasive techniques now available. There is interesting ongoing research on use of nebulization to deliver surfactant.[65, 66]

1.2.1.4 The fetal period: saccular stage (weeks 24–26 to 36)

The transition zone between the canalicular and the saccular stage marks the current limit of viability for preterm birth at around 22–23 weeks' gestation. The ends of the airways form saccules that, throughout this stage, develop into alveolar ducts and alveolar sacs. True alveoli can be seen at around 32 weeks but are often more recognizable at 36 weeks. There is marked expansion of the vascular network with growth of blood vessels and formation of new arteries and veins. Continuing development of the thin blood–air barrier leads to a significantly increased gas exchange area.[62, 64]

1.2.1.5 The postnatal period: alveolar stage (around week 36 to post-term)

The alveolar stage starts a few weeks before term and continues after birth, with over 85% of alveoli formed after birth. Alveoli are small, thin-walled sacs lined by epithelial cells that facilitate gas exchange between inhaled air and blood. The alveolar epithelium consists of type I and type II pneumocytes. Type I pneumocytes cover approximately 90% of the alveolar surface, with their basement membrane fusing with that of capillary endothelial cells to form the blood–air barrier. Type II pneumocytes occupy only up to 10% of the alveolar surface and are mainly located among the type I pneumocytes.[62, 64]

During the alveolar stage of lung development, secondary septation divides alveolar ducts into terminal alveoli, leading to a rapid increase in the number of alveoli, with between 100 million and 150 million alveoli formed at full term.[62, 64] With angiogenesis continuing in parallel, the alveolar stage contributes to further expansion of the gas exchange surface for maximal gas transfer. A pulmonary acinus refers to a gas-exchanging unit, distal to a terminal bronchiole, composed of respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli.

1.2.2 Lung development after birth

In the fetus, gas exchange occurs in the placenta. This changes at birth when gas exchange takes place in the lungs, and the first breath of air marks the beginning of the newborn's life. Although the lungs of a term newborn are functional, they are not fully developed yet. At this stage, the process of airway generations and branching is complete, but not alveolarization. While early structural studies suggested that alveolarization mainly occurs in the first 2–4 years of life,[67] this hypothesis has been challenged by subsequent research in recent years. Current consensus is that alveolarization most likely extends beyond early childhood, as indicated by studies using helium-3 magnetic resonance.[68, 69]

Lung size increases in proportion to body size and is influenced by age, sex, and ethnic origin.[70, 71] Thus, males have larger lungs and a higher number of alveoli than females.[72]

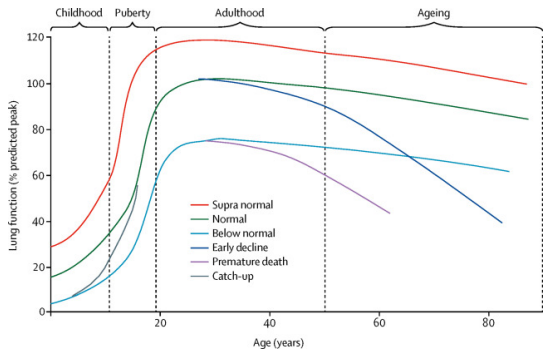
In preterm infants, the lungs must take over from the placenta earlier than normal for gas exchange, i.e. months before lung development is complete. In addition, lifesaving treatment such as mechanical ventilation and hyperoxic exposure (even room air is hyperoxic, compared to the fetal environment) can induce further lung injury and affect normal alveolar growth and development.[63]

1.3 Lung function trajectories over the lifespan

In healthy individuals, lung growth and development, as assessed by lung function tests, follow a distinct pattern (trajectory) over the lifespan. This trajectory can be

divided into three separate phases: (1) a growth phase (from birth to early adulthood); (2) a short plateau phase (lasting for a few years); and (3) a decline phase (characterized by physiological lung ageing). Maximal lung function is usually reached by the age of around 20–25 years, and earlier in women.[73, 74] Birth cohort studies showed that lung function tracks throughout the life course.[74, 75] Importantly, poor lung function at birth (and early childhood) establishes a life-long trajectory of poor lung function. Additionally, lung function trajectories can be influenced by numerous factors such as childhood pneumonia, asthma, underweight, and smoking exposure during the critical windows of lung growth and development, resulting in shifts from a 'normal' to a lower trajectory.[75] Different potential lung function trajectories are depicted in Figure 1.

Figure 1: *Potential lung function trajectories throughout the life course.*



Reprinted from The Lancet Respiratory Medicine, Vol. number 7, Alvar Agusti and Rosa Faner, Lung function trajectories in health and disease, Pages No. 358-64, Copyright (2019), with permission from Elsevier.

1.4 Respiratory sequelae of preterm birth

1.4.1 Respiratory distress syndrome

RDS occurs in a high proportion of preterm infants, although it can also occur in term-born neonates.[76, 77] It was previously known as hyaline membrane disease due to histopathological findings of hyaline membranes containing cellular debris, fibrin, red blood cells, inflammatory cells, and injured epithelium lining the collapsed alveoli.[78,

[79] RDS is now primarily recognized as a disorder of surfactant deficiency together with structural and functional immaturity of gas-exchanging units, resulting in respiratory distress soon after birth.[80] RDS is commonly diagnosed based on a combination of clinical and radiographic features. Male sex and Caucasian ethnicity are associated with a higher risk of RDS,[77] and there is a significantly higher incidence of RDS among infants of diabetic mothers due to delayed lung maturation.[81]

Hypoxaemia resulting from RDS secondary to surfactant deficiency and lung immaturity causes reduced oxygen delivery to peripheral tissues, resulting in production of lactic acid. Thus, respiratory acidosis prevents natural dilatation of the pulmonary vasculature, which can lead to pulmonary hypertension, which, in turn, can further exacerbate the situation. Right-to-left shunting of blood from the pulmonary artery via the ductus arteriosus into the systemic circulation contributes to perpetuating the hypoxaemia and acidosis, thereby establishing a vicious circle that can be difficult to break.[79]

RDS management aims at increasing survival and reducing morbidity, including the risk of bronchopulmonary dysplasia (BPD). European guidelines[82] on prevention and treatment strategies in RDS recommend administering prenatal corticosteroids to all mothers at risk of preterm delivery up to 34 weeks' gestation, judicious control of oxygen supplementation in resuscitation of the newborn, use of CPAP in stabilizing the infant, and reserving intubation to infants who do not respond to positive pressure ventilation via face mask. The guidelines also provide recommendations on surfactant therapy. Further management following stabilization of the infant should focus on supportive care, including maintaining body temperature, initiating intravenous nutrition, and maintaining appropriate blood pressure and haemoglobin (Hb) levels.[82]

1.4.2 Bronchopulmonary dysplasia

BPD is the most common respiratory disease among infants born EP. It was first described by Northway *et al*. in 1967[83] as a disease characterized by intense inflammation and airway damage caused by aggressive mechanical ventilation and oxygen toxicity, now known as 'old BPD'.[84] Advances in NICU care, including more

gentle ventilation, introduction of antenatal steroids, and surfactant treatment, resulting in survival of increasingly more immature infants, has led to the term 'new BPD', which is characterized mainly by acinar dysplasia, rather than by severe inflammatory insult that occurs in 'old BPD'.[85, 86] BPD is a heterogeneous clinical syndrome of lung injury that disrupts the normal development of alveoli and the microvasculature. Despite major improvements in prenatal and postnatal treatment throughout the past five decades since its first description, BPD remains the most common and severe chronic disease among preterm infants, with possible life-long consequences.[87]

Mechanical ventilation, which is mandatory for survival, may also cause lung damage. Influx of neutrophils and macrophages into the alveoli[88] and production of pro-inflammatory cytokines may disrupt lung development in infants with BPD.[89, 90] Long-term use of respiratory support during the neonatal period may exacerbate pulmonary inflammation by inducing the production of reactive oxygen species (ROS)[91] which also promote epithelial cell death in the lungs.[88]

The incidence of BPD increases with decreasing GA and BW, and infants with BW of <1250 g and GA of <30 weeks account for 97% of all BPD cases.[92] The prevalence of BPD among EP-born infants varies widely globally, ranging from around 10% to 68% across different countries.[76, 93, 94] In a nationwide Norwegian study of infants born EP in 1999 and 2000, the incidence of moderate and/or severe BPD was 67% among infants with a GA of 23–25 weeks, and 37% among those with a GA of 26–30 weeks.[95] The large variation in the incidence of BPD is probably due to differences in definitions and variations in management approach and survival rates, as well as in the characteristic of the neonatal units.

1.4.2.1 Risk factors for, and predictors of, bronchopulmonary dysplasia

The pathogenesis of BPD is not fully understood. BPD might be a direct consequence of lung immaturity, as well as of the pathology that caused premature birth.[96] Known risk factors for BPD are low BW and IUGR, male sex, exposure to maternal smoking, genetic factors, and postnatal events related to neonatal intensive care management, such as exposure to high oxygen levels and mechanical ventilation, as well as sepsis.[87, 97]

Preterm birth is the biggest risk factor for developing BPD. However, not every preterm infant will develop BPD, and it is not possible to reliably predict which infant will be affected by the condition.[98, 99] Recent novel technologies involving 'omics'-based approaches that enable genomic, epigenomic, proteomic, microbiomic, and metabolomic analyses have shown promising results in terms of better prediction of the likelihood of BPD among preterm infants.[98, 100] Use of flow–volume loops obtained from ventilator flow data has been suggested to help predict BPD in EP infants.[101] Identification of infants as being 'high risk' for BPD would allow timely and targeted management provided by clinicians.

1.4.2.2 Definition and diagnosis of bronchopulmonary dysplasia

The currently applied definition of BPD, since the beginning of the twenty-first century, is based on the requirement for supplemental oxygen at a postnatal age of 28 days.[102] The severity of BPD can be further graded at 36 weeks post-menstrual age (PMA) as 'mild', 'moderate', and 'severe'. Infants with mild BPD receive oxygen or respiratory support for >28 days but are exposed to room air at 36 weeks PMA. Moderate BPD involves infants requiring <30% supplemental oxygen at 36 weeks PMA, whereas severe BPD occurs when infants require >30% oxygen or positive pressure at 36 weeks PMA.[102] As the actual fraction of inspired oxygen at 36 weeks is often unknown, both moderate and severe BPD are commonly referred to as moderate/severe BPD, while cases involving oxygen requirement at 36 weeks are sometimes simply referred to as BPD.

The correlation between a diagnosis of BPD and long-term pulmonary or neurodevelopmental outcomes has been inconsistent across studies, and predictive values of long-term consequences are low.[103, 104] Moreover, with changes in neonatal care, including increased use of non-invasive respiratory support, often in room air, there is a need of updated definitions to ensure appropriate classification.[105] The National Institute of Child Health and Human Development (NICHD) proposed in 2018 refinements to the existing BPD definitions and takes into consideration new modes of non-invasive ventilation.[104] The proposal includes a grading system (grades I, II, and III) based on persistent parenchymal lung disease, radiographic confirmation

of parenchymal lung disease, and requirement for different oxygen levels via invasive IPPV, nasal CPAP (n-CPAP), nasal IPPV (NIPPV), or nasal cannula at 36 weeks PMA.

1.4.2.3 Management of bronchopulmonary dysplasia

The most effective strategy to prevent BPD is to avoid EP birth. However, all efforts aim to achieve this goal, the approach to management also focuses on treatment strategies to minimize long-term damage, including prevention of IUGR.[6, 106] Antenatal corticosteroid therapy[107] has been shown to reduce neonatal mortality and complications, although it does not prevent BPD.[42] Non-invasive ventilation, including n-CPAP or NIPPV, is often used, instead of intubation and mechanical ventilation, to avoid lung injury,[108, 109] but with no clear reduction in BPD incidence observed so far.[110, 111] Surfactant therapy has not been demonstrated to have a definite impact on BPD incidence.[112, 113] However, recent studies have reported less invasive surfactant administration (LISA) of surfactant therapy resulted in reduced BPD incidence.[114, 115]

Further, it is important to prevent neonatal infections, as these are a known risk factor for BPD.[116] Postnatal steroid treatment (mainly with dexamethasone) has been trialled as a preventive strategy and shown to be associated with improved lung outcomes. However, due to important adverse effects, such as increased incidence of cerebral palsy,[117-119] hyperglycaemia, hypertension, and growth failure, postnatal steroids are not routinely used.[117, 120] Trials of postnatal hydrocortisone therapy, aerosolized budesonide, or budesonide mixed with surfactant have demonstrated some benefits,[121-123] indicating these could be potential future treatment options.

As inflammation plays a significant role in the pathogenesis of BPD, research is under way investigating possible therapeutical targets to prevent lung inflammation, including inhibitors of leukotrienes and cytokine production, as well as cellular therapy with immune modulating and pro-regenerative effects.[97, 124, 125]

Identification of infants at particular risk of developing BPD is of crucial importance and the hope is to be able to provide innovative, combined, and tailored therapy in order to achieve the best outcomes.

1.4.3 Outcomes of previable preterm premature rupture of membranes

PV-PPROM poses a great risk to both mother and child. The mother is exposed to the risk of possible preterm labour, chorioamnionitis, endometritis, cord prolapse, and placental abruption.[29, 30, 126] The most feared infant complications of PV-PPROM are chorioamnionitis and EP birth, pulmonary hypoplasia, respiratory failure, IVH, limb contractures, and death.[126] The combination of pulmonary hypoplasia and limb contractures occurs due to a lack of amniotic fluid and may clinically present similarly to Potter's syndrome.[127] Pulmonary hypoplasia occurs in approximately 50% of mid-trimester PPROM cases diagnosed before 19 weeks' GA.[128]

A recent study found that around 28% of neonates born following PPROM prior to 23 weeks' GA survived to NICU discharge.[129] In a systematic review on maternal and neonatal outcomes following PV-PPROM, Sim *et al*.[130] reported an overall live birth rate of 63.6% and a survival-to-discharge rate of around 45%, in agreement with two retrospective studies.[126, 131] The review found that the majority of early neonatal deaths (within 24 hours post-birth) were associated with pulmonary hypoplasia, severe IVH, and neonatal sepsis.[130] The proportion of neonates that survived to discharge with no morbidity was <30%, whereas the proportion that survived with composite morbidity ranged between 12.5% and 85.7%.[130] Composite morbidity was, in most of the studies included in the systematic review, defined as the presence of one or more of the following: IVH grade III or IV, pulmonary hypoplasia, periventricular–intraventricular haemorrhage (PIH), periventricular leukomalacia (PVL), severe necrotizing enterocolitis (NEC), ROP grade 3 or 4, BPD, sepsis, and/or neonatal death.[130]

Only a few studies have reported on outcomes in PV-PPROM cases beyond the neonatal period and they only included follow-up into early childhood.[132-134] Generally, neonatal and early childhood respiratory and neurological outcomes have been found to be worse in infants delivered following early PPROM (i.e. PPROM occurring before 25 weeks' GA), compared to those delivered following late PPROM, in follow-up studies of children aged between 18 months and 4 years, with an increased rate of long-term sequelae of 25–50% in those surviving to discharge.[131-135]

There is a general paucity of published data on later childhood outcomes, in particular cardiopulmonary outcomes. Long-term consequences of PPROM and PV-PPROM are largely unknown, thus warranting further research in this area.

1.4.4 Pulmonary hypertension related to premature birth

Pulmonary hypertension (PH) is defined as increased blood pressure in the pulmonary arterial system. This is a normal and necessary state during fetal life when the placenta serves as the gas-exchanging organ and the fetal lungs are fluid-filled, with most blood shunted past the lungs through the ductus arteriosus. Thus, pulmonary artery resistance is very high, resulting in pulmonary pressures being similar to systemic pressure in the fetus.[136]

The first breath taken by a neonate at birth allows air to enter the alveoli, inducing a dramatic physiological process whereby pulmonary artery resistance is significantly reduced secondary to increased oxygen tension, together with a rise in pulmonary blood flow. The ductus arteriosus and foramen ovale subsequently close, and the pulmonary circulation reaches maturity as a high-flow, low-resistance, and low-pressure system.[137] However, if the normal cardiopulmonary transition at birth fails, right-to-left shunting continues across the ductus arteriosus and foramen ovale, while the high resistance and pressure in the pulmonary circulation persist, resulting in persistent PH.[136, 137]

Neonatal PH can be caused by multiple factors, including congenital heart disease, developmental pulmonary vasculature abnormalities, and persistent PH of the newborn (PPHN).[138, 139] Paediatric PH is understudied, with research mostly focusing on PH in adult populations. The mechanisms underlying paediatric PH are poorly understood, and specific treatment of PH in paediatric patients is lacking.

PH is increasingly recognized as an important cause of morbidity and mortality in premature infants, even in the 'surfactant therapy' era,[138] and guidelines recommend that all infants with BPD undergo echocardiographic assessment for PH at 36 weeks PMA.[140, 141] A recent systematic review found that up to approximately 40% of infants with BPD developed PH, with the highest prevalence seen in those with severe BPD.[142] PH has also been observed in EP-born infants without BPD.[142]

Researchers also reported increased rates of PH with increasing survival among 23- to 26-week preterms.[138, 140, 142] Pulmonary vascular disease is poorly studied in EP-born infants without BPD, and the true incidence of PH after EP birth is not known. Thus, a considerable number of infants with PH within the EP population might be undiagnosed.[142] A Swedish study published in 2019 indicated that preterm birth was associated with PH later in life.[143]

The pathophysiology of PH in premature infants is not fully understood. It is most likely due to a combination of factors, including disruption of normal lung and vasculature development, resulting in a reduced cross-sectional area for blood flow and elevated pulmonary vascular resistance (PVR), which alters vasoreactivity and causes structural remodelling.[140] Both prenatal (e.g. IUGR, pre-eclampsia, chorioamnionitis) and postnatal factors (e.g. mechanical ventilation, oxidative stress related to both hypoxia and hyperoxia, infection, haemodynamic overcirculation) are likely involved in the pathogenesis of PH.[137, 140, 144, 145] Moreover, oligohydramnios, PPROM, and pulmonary hypoplasia have been associated with the development of PH,[140, 146] although a systematic review and meta-analysis published in 2018 found no association between PPROM and PH.[142] Prolonged oligohydramnios is associated with pulmonary hypoplasia, with decreased alveolar count and reduced size of the pulmonary vascular bed with abnormal vascular muscular development.[147] It has been hypothesized that infants born following PPROM may have a transient deficiency of endogenous nitric oxide (NO), due to defective NO production and/or signalling, that leads to the development of PH.[148]

1.4.4.1 Diagnosis of pulmonary hypertension

Historically, guidelines on paediatric PH have been based on the same definitions used for adult PH, i.e. resting mean pulmonary artery pressure of ≥25 mmHg. This definition specifically applies to children aged ≥3 months.[138, 141] In younger infants, a systolic pulmonary artery pressure of 36 mmHg is commonly considered as the upper limit of normal.[141] Updated paediatric guidelines from 2019 recommend indexing the PVR in relation to the body surface area—the PVR index (PVRI)—to assess for the

presence of pulmonary vascular disease (PVD), defined as PVRI ≥3 Wood units (WU)·m^2.[149]

Paediatric PH is classified into different groups based on the underlying cause, according to the Paediatric Task Force of the 6th World Symposium on Pulmonary Hypertension (WSPH). PH due to developmental lung diseases, including BPD, is classified as group 3.5.[138]

Cardiac catheterization enables direct measurement of the pulmonary artery pressure and is the gold standard for diagnosing PH.[138, 141] However, it is an invasive procedure performed under general anaesthesia. Therefore, transthoracic echocardiography is more commonly used in children.[141] Echocardiography estimates the systolic pulmonary artery pressure using the tricuspid regurgitation jet velocity (TRJV) and a modified Bernoulli equation to determine the pressure gradient between the right ventricle (RV) and the right atrium. In the absence of tricuspid stenosis or other structural anomalies, RV pressure and pulmonary artery pressure are equal during systole. Qualitative evidence of PH includes flattening of the interventricular septum and hypertrophy/enlargement of the right heart chambers.[144]

1.4.4.2 Management of pulmonary hypertension in neonates

Before initiating treatment targeted specifically at PH, guidelines recommend focusing on recognizing and treating underlying causative factors. This includes assessment for hypoxaemia, aspiration, structural airway abnormalities, and any potential need of changes to respiratory support.[141]

The aim of PH treatment is to achieve selective pulmonary vasodilatation, either by stimulating the NO or prostacyclin pathways (to promote vasodilatation and inhibit smooth muscle proliferation) or by blocking the endothelin pathway (endothelin-1 is a potent vasoconstrictor and is upregulated in PH). Inhaled NO (iNO) is a rapid and potent vasodilator and is currently the first-line therapy in infants with PH. Other possible therapies include phosphodiesterase type 5 (PDE-5) inhibitors, prostacyclin, and endothelin receptor antagonists (ERAs).[140, 141, 144, 150]

1.4.5 Pulmonary structural changes following extremely preterm birth

There is scant evidence on lung structure and development of peripheral lung structures following EP birth. Most data come from histological studies, animal models, or post-mortem examinations of infants who died primarily of severe lung disease. Studies of survivors of EP birth, particularly post-infancy in clinically stable subjects, are lacking, and generalizability of existing data is not necessarily possible. Histopathological data are mostly on infants and young children aged <3 years,[151-156] with some few exceptions.[157-161]

The severe pathological changes of fibrosis, atelectasis, smooth muscle hypertrophy, inflammation, and hypertensive vascular lesions observed in infants with 'old' BPD have evolved with the advent of modern neonatal intensive care and lower GA of preterm infants. Histopathological evidence indicates disruption of normal development of alveolar units, characterized by loss of normal alveolar structure, alveolar simplification, and other more subtle lung abnormalities in smaller and more immature infants. Findings in both the pre- and post-surfactant and prenatal steroid eras in the treatment of 'new' BPD are similar,[162-164] and it has been proposed that severity variation may be influenced by the duration of ventilatory support, postnatal lung infections, and nutritional status.[163] In addition, impaired acinar development is a consistent histopathological abnormality found in BPD, demonstrated by reduced alveolar numbers, reduced secondary septation, and microvascular growth simplification.[162, 164-166] Of note, the introduction of surfactant treatment has resulted in fewer infants developing fibrosis, and with decreased severity.[154-157]

A few case reports have been published on lung pathology in older children and young adults born EP.[158-161] Although there is some degree of variation, post-mortem studies and lung biopsies from preterm-born individuals aged 12–34 years revealed signs of persistently enlarged, simplified alveoli, various degrees of septal fibrosis, and abnormal vasculature.[158-161] However, all subjects had severe pulmonary disease, so these findings are not generally transferable to all premature survivors. Analysing lung tissue from survivors of EP birth with BPD, but without clinical signs of pulmonary disease, or from EP-born subjects who died from non-pulmonary causes should therefore be a high research priority in future.

1.4.5.1 Animal models

Animal models of inhibited alveolar–capillary membrane development, including mice,[167] rats,[168] rabbits,[169] lambs,[170, 171] pigs,[172] and baboons,[173] have been developed to improve our understanding of lung development following preterm birth and the pathogenesis of BPD, as well as to evaluate possible therapies. While preterm birth infants often are exposed to various insults, often in combinations, such as hyperoxia, ventilator-associated trauma, comorbidities, and infections, most animal models assess only single factors. It is therefore extremely difficult establish an experimental animal model that would mimic the different possible permutations of risk factors that could result in lung pathology in EP-born infants.[174] An ideal animal model that replicates the exact lung features present in EP-born humans does not exist, resulting in huge discrepancies between actual human clinical settings and the *in vivo* settings of animal models.

1.4.6 Long-term pulmonary outcomes of extremely preterm birth

General pulmonary outcomes following EP birth will be discussed briefly below. Gas exchange and studies measuring lung diffusing capacity in relation to EP birth will be discussed later (see Section 2.1.3.1).

1.4.6.1 Pulmonary outcomes in childhood

Children born EP, in particular those with BPD, are at higher risk of rehospitalization in their first few years of life, most commonly due to respiratory illness.[175-182] However, the rate of hospital readmissions decreases in late childhood.[176, 179]

Preterm-born children present with more respiratory symptoms, such as wheeze, cough, and asthma-like symptoms, and report higher use of asthma medications, compared to term-born children.[176, 182-184] In a meta-analysis of birth cohort studies, children born before 28 weeks' gestation were found to have the highest risk of preschool wheezing (odds ratio (OR) 3.87, 95% confidence interval (CI) 2.70–5.53) and school-age asthma (OR 2.92, 95% CI 1.84–4.62).[185]

Pulmonary function tests generally show reduced expiratory flows and volumes among preterm-born infants, with greater deficits among those with BPD.[178, 184, 186-188] Moreover, the residual volume (RV)-to-total lung capacity (TLC) ratio tends to be increased, indicating air trapping.[189, 190] High-resolution computed tomography (HRCT) reveals pulmonary structural changes persisting into school age in children born preterm with BPD.[191, 192]

1.4.6.2 Pulmonary outcomes in adolescence and early adulthood

EP-born adolescents and young adults are more prone to asthma-like symptoms, wheeze, and cough.[193-198] Those with a history of BPD have persistent airflow obstruction, with lower forced expiratory volume in 1 second (FEV_1) and higher RV.[182, 187, 194, 195, 199-202] Although there is some variability, overall, there seems to be little difference in FEV_1 in individuals born prior to the surfactant era, compared to those born when surfactant therapy has become available. Of note, however, there is variation in reports of lung function outcomes later in life among EP-born cohorts, with some studies reporting improvements in lung function over the years and others showing worsening in pulmonary outcomes in later life.[186, 187, 203]

Further, radiological pulmonary abnormalities have been reported in survivors of EP birth, including reduced lung attenuation, bronchial wall thickening, and even emphysema.[192, 197, 204, 205]

1.4.6.3 Pulmonary outcomes in adulthood

Most long-term studies have examined subjects in their twenties. As the oldest survivors of EP birth are now approaching their forties, data on pulmonary outcomes over the lifespan are yet to be obtained. It is hoped that valuable information on respiratory health in late adulthood in those born EP will become available in the years to come.

It has been shown that respiratory symptoms and lung function impairment persist into the mid-twenties.[206-208] In one study, very low-birthweight (VLBW) adult subjects, with a mean GA of 29.2 weeks, had a higher incidence of airflow obstruction, gas trapping, reduced gas exchange, and increased ventilatory inhomogeneity,

compared to controls.[209] Spirometry variables for the EP cohorts included in this thesis have been demonstrated to track in parallel, but significantly below the trajectories of matched term-born controls to 35 years of age.[208]

1.4.6.4 Association between premature birth and chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is now the third leading cause of death worldwide,[210] and a major cause of disease burden.[211] COPD is characterized by persistent respiratory symptoms and airflow limitation. It is defined by an FEV_1-to-forced vital capacity (FVC) ratio of <0.70 and is further classified according to severity, based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) grading system (GOLD grades 1–4) using post-bronchodilator FEV_1 measurements.[212] While COPD is mainly regarded as a smoking-related disease, it is increasingly recognized that, in some cases, it can be related to events that occurred early in life. For instance, factors affecting lung growth during gestation and in childhood may increase the risk of developing COPD later in life.[213-215] There is also an association between BW and lung function in adulthood,[216, 217] as well as a synergistic interaction between smoking and infant respiratory infection which can influence adult lung function trajectories.[218] Long-term follow-up studies of children born prematurely have shown 'tracking' of lung function into early adult life, further supporting the hypothesis that impaired lung function present shortly after birth is a risk factor for negative pulmonary outcomes in adult life.[73] As previously mentioned, evidence exists that alveolarization continues into adolescence in children with a history of premature birth.[68] Therefore, this suggests that even events occurring later in early adolescence can affect long-term lung function trajectories. It is likely that the additive effects of premature birth and early life respiratory insults impact both short- and long-term respiratory health and function in EP-born children diagnosed with BPD.[215]

A high proportion of babies born prematurely now survive infancy and even though they often suffer from more respiratory symptoms, most gradually become less symptomatic throughout childhood. However, while respiratory symptoms tend to

fade, lung function impairments seem to persist and many do not reach their expected plateau of lung function at around the age of 20–25 years.[207] Hence, the starting point for the normal physiological age-related decline in lung function in these individuals may be at a lower and suboptimal level. Whether the rate of decline in lung function with age will parallel that of healthy individuals or will be accelerated is unknown. Both an accelerated decline in lung function and failure to reach the expected peak lung function are known risk factors for developing COPD.[219] Physiological age-related decline in lung function may therefore predispose individuals born prematurely to a COPD-like phenotype, in contrast to most healthy non-smoking term-born individuals who have large reserves.[220] Spirometry data from childhood to the mid-30s for extremely preterm-born cohorts, including the two cohorts included in this thesis, have shown that one in three met the spirometry criteria for the COPD diagnosis[208]. Therefore, clinicians should have a low threshold for suspicion of respiratory symptoms and performing lung function measurements when managing EP-born individuals.

2 Special introduction: Gas exchange and lung diffusing capacity following preterm birth

2.1 Gas exchange and lung diffusing capacity

2.1.1 General bacground

Gas exchange involves passive diffusion of oxygen (O_2) in inspired air from the alveolar spaces into the bloodstream, with concurrent diffusion of carbon dioxide (CO_2) from the bloodstream into the alveolar spaces, which is then exhaled.

According to Fick's law,[221] passive diffusion of gases is governed by the: (1) available surface area; (2) diffusion distance; and (3) concentration gradient. An optimal lung should therefore have the largest possible surface area, a thin diffusion barrier, and a steep concentration gradient to facilitate gas exchange. The main goal of lung development is to generate an organ that satisfies these criteria.

The lungs consist of hundreds of millions of alveoli which provide an enormous surface area for efficient gas exchange. The alveolar–capillary membrane is normally very thin and permeable to many gases, including O_2 and CO_2, with only a small distance across which gas molecules diffuse for effective gas diffusion. The partial pressure of O_2 (pO_2) is higher in the alveolar air spaces than in pulmonary capillaries, resulting in passive diffusion of oxygen from the alveoli through the alveolar–capillary membrane into the capillaries along the partial pressure gradient. By contrast, the lower partial pressure of CO_2 in alveolar air spaces results in diffusion of CO_2 in the opposite direction. The structure of the blood–air barrier in the human lung is thus extremely well suited for diffusion according to Fick's law.

2.1.2 Diffusing capacity of the lung for carbon monoxide

While lung biopsy would provide the most accurate data in the study of long-term consequences of preterm birth on alveolar–capillary membrane development through direct histological examination of biopsy samples and alveolar counts, it is a highly invasive procedure that would be ethically challenging to perform for research purposes in otherwise relatively healthy individuals. Clinical measurements of gas exchange, including the diffusing capacity of the lung for carbon monoxide (DL_{CO}),

serve as a surrogate marker of alveolar–capillary membrane growth. DL_{CO} is also known as the transfer factor for carbon dioxide (TL_{CO}). Traditionally, the term TL_{CO} is mostly used in European literature, and DL_{CO} most commonly in American literature, although the terms are used interchangeably and DL_{CO} seems to be slightly favoured nowadays. The American Thoracic Society (ATS) and the European Respiratory Society (ERS) task force for standardization of lung function testing[222] use the term DL_{CO}, which therefore will be used in this thesis.

Carbon monoxide (CO) is as the test gas, substituting for O_2 as the exact concentration of O_2 in the blood returning to the lungs from tissues is highly variable and thus difficult to measure. CO can be assumed to be absent in blood; it has the same diffusion properties as O_2 and binds to Hb with high affinity, resulting in nearly all CO molecules binding to Hb. The diffusing properties of the blood–air barrier limit the amount of CO diffusing out of the alveoli—in other words, CO is a diffusion-limited gas.

DL_{CO} measures the conductance of gas transfer from inspired gas to red blood cells in the pulmonary capillaries. It is a measurement of the volume of CO transferred from alveolar gas to capillary blood per unit time per unit of driving pressure of CO. DL_{CO} is measured in $mmol \cdot min^{-1} \cdot kPa^{-1}$ (ERS) or in $mL \cdot min^{-1} \cdot mmHg^{-1}$ (ATS).

Transfer of CO from inhaled test gas to blood in the pulmonary capillaries occurs in six steps:[222]

1. Bulk flow delivery of CO to the airways and alveolar spaces
2. Distribution of CO in the alveolar ducts, air sacs, and alveoli
3. Diffusion of CO across the alveolar–capillary membrane
4. Distribution of CO in the alveolar–capillary plasma
5. Diffusion of CO across the red cell membrane and within the red blood cell
6. Chemical reaction of CO with Hb in the red blood cell.

The process can be simplified into two different conductance components: (1) membrane conductivity (D_M), representing CO diffusion across the alveolar–capillary membrane into the red blood cell; and (2) the vascular component of diffusion, represented by chemical binding of CO to Hb (θ) and the volume of alveolar–capillary blood (V_C). Since these two conductance components are in series, the relationship

between the membrane component and the vascular component can be expressed by the equation below, as proposed by Roughton and Forster in 1957:[223]

$$\frac{1}{\mathrm{DL_{CO}}} = \frac{1}{D_\mathrm{M}} + \frac{1}{\theta V_\mathrm{C}}$$

As mentioned earlier, diffusing capacity is, in fact, conductance; however, conductances cannot be added. Since resistance is the reciprocal of conductance, the factors in the equation are therefore inversed.

The relative importance of each of these two components (i.e. D_M and θV_C) can be determined separately by measuring $\mathrm{DL_{CO}}$ at two different $\mathrm{O_2}$ concentrations. The rate of chemical reaction of CO with Hb (θ) can be altered by changing $p\mathrm{O_2}$ in the alveolar gas. With increasing $p\mathrm{O_2}$ in the alveoli, CO competes with $\mathrm{O_2}$ for Hb binding, reducing the θ component. Thus, $1/\mathrm{DL_{CO}}$ is plotted against $1/\theta$ at the two different $\mathrm{O_2}$ concentrations. The slope of this relationship is $1/V_\mathrm{C}$, and the intercept is $1/D_\mathrm{M}$. By changing the θ values, it is possible to measure separately the diffusing capacity caused by the physical process of diffusion (D_M) and the resistance caused by the rate of the reaction of uptake of CO by Hb.

Although measurements at two different $\mathrm{O_2}$ tensions are not performed routinely in clinical settings, they may be useful in determining the pathophysiological mechanism of reduced $\mathrm{DL_{CO}}$ in different settings.

2.1.2.1 The single-breath technique

The most widely used method of measuring the diffusing capacity of the lung is the single-breath (SB) technique. The method was developed by Marie Krogh[224] over a century ago and is based on measuring the difference in CO concentration between inhaled and exhaled air. The SB technique has been standardized by the ERS and ATS, with reference values for healthy subjects, and is the most commonly used method for measuring $\mathrm{DL_{CO}}$ in clinical studies. This section will focus on the theoretical background to the technique, and its practical implementation will be described in Section 4.3.4.1.1.

The SB technique determines CO uptake from the lung over a breath-holding period approaching TLC by measuring the difference in CO concentration between inhaled and exhaled air. The rate at which CO is removed from the alveolar space increases exponentially and is directly proportional to the breath-holding time, and the slope at which the CO concentration declines over the breath-holding time is known as the rate constant for CO uptake from alveolar gas (K_{CO}, or the transfer coefficient of the lung for CO). DL_{CO} is the product of K_{CO} and the alveolar volume (V_A) that is accessible for gas exchange. After correcting for partial pressure of CO in the test gas, DL_{CO} is expressed as alveolar CO uptake per minute per kilopascal. DL_{CO} can also be expressed by the equation:

$$\frac{[V_A \cdot K_{CO}]}{Pb^*} = DL_{CO}$$

where Pb* is the barometric pressure minus the water vapour pressure at 37°C in alveolar gas.[225]

When performing the SB-DL_{CO} technique, the test subject inhales a gas mixture containing a low concentration of CO (typically 0.3%) and an inert gas (usually helium, methane (CH_4), or neon), as well as O_2, balanced with nitrogen. The inert gas is used to determine the alveolar volume (V_A) based on its known concentration in inhaled air and by measuring its concentration in exhaled air. CH_4, which is essentially insoluble and therefore cannot diffuse across the alveolar–capillary membrane, was used in the studies presented in this thesis. Because V_A is determined from measurements of the inert gas, it only measures those areas of the lung that communicate with the mouth, thus excluding areas with trapped gas from measurement.[226] In normal subjects, V_A is within 10% of the TLC, with a mean *V*A-to-TLC ratio (both men and women combined) of 93.5% ± 6.6 (1 standard deviation (SD)).[225]

K_{CO} expressed as DL_{CO}/V_A is sometimes, albeit misleadingly, referred to as 'DL_{CO} corrected, or adjusted, for alveolar volume'.[225] The relationship between lung volume and CO uptake is complex and non-linear. Therefore, K_{CO} calculation cannot

be used as a simple method to normalize DL_{CO} for volume.[222] K_{CO} reflects the efficiency of alveolar CO uptake at a given volume, and it is important to determine both DL_{CO} and K_{CO} to understand clearly the clinical implications of an altered diffusing capacity. For example, a decrease in DL_{CO} can be the result of reduced K_{CO} (determined by D_M and V_C) or decreased V_A.[227] It is therefore important to report all these variables to enable correct data interpretation.

Handling of the test equipment and measurements should be carried out according to standard recommendations.[222] The method is more complex to perform than, for example, a regular spirometry measurement, particularly due to the requirement for long breath-holding duration (10 ± 2 seconds). The SB technique is demanding, both for the test subject who needs to exert greater effort and for the technician giving the instructions, and may be technically challenging to perform in children, particularly in the presence of cognitive, sensory, or physical impairments.

2.1.2.2 The intra-breath technique

An alternative to the SB method is the intra-breath (IB) technique, which was first described by Newth *et al*.[228] The technique may be easier to perform as it does not require a long breath-holding duration. It may therefore be a useful alternative if the SB technique fails, e.g. in younger children and dyspnoeic patients. A special rapid infrared analyser is used to measure CO uptake throughout a single exhalation. The IB technique is described in detail by Wilson *et al*.[229]

Both the IB and SB techniques are performed similarly up to the point of breath-holding. In the IB method, the test subject inhales fully, and thereafter fully exhales smoothly and slowly at a constant rate. Gas concentrations in the exhaled air are then subsequently measured to calculate DL_{CO}.

Although the IB technique may be potentially useful in certain patient groups, it is not very commonly used. Main reasons include lack of technique standardization and recommendation by both ERS and ATS[230] to use the SB technique DL_{CO} measurement.

The practical implementation of the IB method will be described in detail in Section 4.3.4.2

2.1.2.3 Factors influencing gas exchange in health and disease

The diffusion process in the human lung may, in theory, be challenged in four ways: (1) thickening of the blood–air barrier; (2) physical exertion; (3) reduced partial pressure of the gas across the blood–air barrier; and (4) reduced binding of CO to Hb. This section describes various situations and conditions that can affect diffusion through these four factors.

At resting heart rate, blood normally passes through the pulmonary capillaries within approximately 1 second. O_2 diffusion commences within 0.3 seconds, which means a healthy person has large reserves for diffusion at rest. During intense physical activity, the cardiac output increases greatly. The transit time of blood through the pulmonary capillaries is therefore much shorter, resulting in less time for binding of O_2 to Hb, thus challenging the gas exchange system. At high altitudes, the lower O_2 fraction in inspired air causes a decrease in pressure gradient, which challenges the

system even further.[231] In anaemia (i.e. reduced Hb concentration), there is reduced binding of CO to Hb, resulting in decreased DL_{CO}. ERS/ATS guidelines recommend for anaemia to be corrected.[222]

Males have higher DL_{CO}, compared to females.[222] Physiological changes affecting D_M or V_C will also influence DL_{CO}, such as Müller manoeuvre (inspiratory efforts against a closed glottis) and supine position where more alveolar capillaries are recruited, along with increased dilatation, which results in increased V_C. By contrast, the Valsalva manoeuvre (expiratory effort against a closed glottis) may reduce V_C.[232] Elevated carboxyhaemoglobin (COHb) concentrations related to tobacco smoking reduce the number of available Hb binding sites and increase CO back-pressure in capillary blood, thereby reducing DL_{CO}. Test subjects should refrain from smoking on the day of undergoing the DL_{CO} test and a correction for CO back-pressure must be made for recent smoking.[222] In addition, DL_{CO} is associated with diurnal variation, with lower DL_{CO} throughout the day. This phenomenon is not fully understood, and some have suggested that it may be due to changes in CO back-pressure and diurnal variation in Hb.[222, 233] Moreover, DL_{CO} varies throughout the menstrual cycle in females, with the highest values observed just before the onset of menses.[234]

Diffusion may be affected by disease. Pathological conditions involving alveolar destruction, such as emphysema and cystic fibrosis and alpha-1-antitrypsin deficiency where alveoli are destroyed by the enzyme neutrophil elastase, may result in a reduced surface area for diffusion, and hence reduced DL_{CO}. Pathological processes that cause increased thickness of the normally thin alveolar–capillary membrane (0.1–0.5 μm), such as fibrosis and alveolitis, result in a longer diffusion distance, and consequently reduced DL_{CO}. Most interstitial lung diseases, such as idiopathic pulmonary fibrosis (IPF) and sarcoidosis, and drug-induced pulmonary toxicity (e.g., methotrexate, nitrofurantoin, amiodarone, bleomycin) are associated with reduced DL_{CO}. Conditions such as anaemia, pulmonary embolism, and PH affect the vascular component of diffusion and thus also may affect DL_{CO}.[226, 235-237]

Reduced DL_{CO} has also been demonstrated in children with type 1 diabetes[237] and Crohn's disease,[238] as well as in childhood leukaemia survivors.[239] Even though these results need to be replicated, nevertheless they indicate that diffusing capacity

testing could be considered also in patients with non-respiratory diseases. Measurement of lung diffusing capacity is non-invasive, relatively widely available, and reasonably affordable, and may provide clinicians with valuable information that could prove useful in understanding the full clinical picture of their patients' condition for optimal treatment.

Even though most diseases are known to reduce DL_{CO}, some conditions may also result in increased DL_{CO}. The most common cause of elevated DL_{CO} is obesity, likely due to increased pulmonary capillary blood volume.[240] Other conditions associated with increased DL_{CO} include polycythaemia (increased Hb), alveolar bleeding (e.g. Wegener's granulomatosis, Goodpasture syndrome), and increased lung perfusion (e.g. left-to-right intracardiac shunting).[240,241] The literature also describes increased DL_{CO} in some asthma patients, possibly due to increased perfusion of the apical regions of the lungs.[242]

2.1.2.4 Normal trajectory of DL_{CO} over the lifespan

Several cross-sectional studies have assessed DL_{CO} in different age groups. While research has included mostly adult participants, a few studies have focused on children in the past two decades.(238-243)

Rosenthal *et al*. measured DL_{CO} in 772 children from the United Kingdom aged 4–11 years, of whom >97% were reported to achieve satisfactory SB measurements.(239) The authors found a near-linear positive relationship between DL_{CO} and height in girls, with no obvious effect of puberty. In boys, there was a sharp discontinuity in the relationship between DL_{CO} and height at around 165 cm, after which DL_{CO} accelerated with increasing height. It is possible that the larger difference of DL_{CO} seen with increasing height in boys could be related to a disproportionate increase in thoracic height, compared to standing height, and, in contrast to girls, there is also an increase in thoracic width. The authors therefore suggested that pre-pubertal and post-pubertal children should be assessed separately.(239)

More recently, large studies in Caucasian children aged 5–19 years demonstrated that DL_{CO} is dependent on height and there is a significant age-dependent effect of sex, particularly in older children and adolescents.(241, 242) This is

probably related to different pubertal effects on somatic and lung growth in males and females. One study reported a decrease in the DL_{CO}-to-V_A ratio with increasing height, indicating that growth of lung parenchyma in this age group occurs mainly via size increase of existing alveoli.[242]

All the above-mentioned studies are cross-sectional. Ideally, longitudinal studies following children over time would help to derive more accurate reference values. While a few longitudinal studies of DL_{CO} in adult populations have been published, longitudinal data on DL_{CO} in children are lacking. Results from longitudinal studies in adult populations indicate a small constant rate of decline of DL_{CO} between the age of 20 and 40 years, followed by an accelerated decline thereafter, independent of tobacco smoking status.[244, 245] A Norwegian study[246] found a mean change in DL_{CO} of −0.025 mmol·min^{-1}·kPa^{-1} per year in the age span from 18 to 72 years and, in agreement with two other studies,[244, 245] an accelerated decline with increasing age. However, in contrast to the two other studies, the Norwegian group found smoking as a predictor for a more rapid decline in DL_{CO}, as well as a dose–response relationship between accumulated exposure to tobacco smoking and the rate of decline.[246]

International sex-specific reference values for DL_{CO} in Caucasians were published in 2017,[247] with an update released in October 2020, as it was found that the reference equations resulted in exceptionally low *z*-scores in females with low DL_{CO}.[248] The reference equations include age and height, with a negative coefficient for age, showing an estimated decrease in DL_{CO} with increasing age. Based on the graphs shown in Figures 1 and 4 from the Global Lung Function Initiative (GLI)'s publication in 2017,[247] it appears that predicted DL_{CO} reaches a maximum at around age 20–25 years, and slightly earlier in females. The population age ranged from 4.5 to 91 years, which makes the reference equations applicable to paediatric populations. Based on the observed variability of DL_{CO}, 0.5 *z*-scores were identified as the threshold for a physiologically relevant difference. This corresponds to 0.3–0.8 mmol·min^{-1}·kPa^{-1} or approximately 10% relative change in DL_{CO}.[247] However, the individual variability of DL_{CO} values was greater early in life and towards the end of life, with a coefficient of variation (CV) of around 20% in the youngest children and falling to around 15% in the oldest adolescents (data from Figure 2 in the GLI all-age

reference values for DL_{CO}).[247] Clinicians should bear in mind this greater variability seen in paediatric populations when interpreting DL_{CO} data from children and adolescents.

2.1.2.5 Repeatability of single-breath DL_{CO}

The variability of the SB-DL_{CO} is influenced by both technical and physiological factors. Variability can be reduced by strictly following the standardized protocol.[222] However, clinicians should always bear in mind inter-laboratory and intra-individual variability when interpreting results, as true DL_{CO} changes must be distinguished from those caused by procedural or normal physiological variability in order to monitor patients over time. Physiological and pathological factors that may affect gas exchange in the lungs are discussed in Section 2.1.2.3. Repeatability of a test describes intra-session variability on repeated testing when test conditions are not changed. According to the ATS/ERS guidelines for DL_{CO} measurements published in 2005,[230] two tests are required that are either within 1 $mmol \cdot min^{-1} \cdot kPa^{-1}$ (or 3 $mL \cdot min^{-1} \cdot mmHg^{-1}$) of each other or within 10% of the highest value. The repeatability requirement was updated in the 2017 guideline, which states that at least two acceptable manoeuvres should be within 0.67 $mmol \cdot min^{-1} \cdot kPa^{-1}$ (or 2 $mL \cdot min^{-1} \cdot mmHg^{-1}$) of each other.[222] Studies in adult populations[249, 250] have concluded that the majority of individuals are able to meet the reproducibility criteria set in the 2005 standardization protocol,[230] and over 90% fulfilled the criteria also when the repeatability requirement was changed to a difference of 0.67 $mmol \cdot min^{-1} \cdot kPa^{-1}$ between the tests.[249] This implies that the adjustment to the reproducibility criteria made in 2017[222] is therefore likely appropriate. However, a possible problem with the updated criteria, where the criteria on percentage difference between tests was taken out, could lie in the fact that an absolute difference of 0.67 $mmol \cdot min^{-1} \cdot kPa^{-1}$ may represent a relatively large percentage difference in children, compared to adults, as children typically have lower DL_{CO} values than adults.

One study of intra-session repeatability of DL_{CO} in children, including subjects with cystic fibrosis, athletes, and healthy controls, demonstrated a CV of around 2.5%.[251] To our knowledge, there were no available data on reproducibility in

children prior to the publication of the study presented in Paper I that is included in this thesis. Reproducibility of the somewhat complex lung diffusing capacity measurements may be influenced to an even higher degree in EP-born subjects due to a higher prevalence of neurosensory, cognitive, and behavioural impairments in this patient group.[252-254]

2.1.3 Gas exchange following extremely preterm birth

EP birth occurs in the second trimester of pregnancy when the lungs are still in the canalicular or saccular stage of development. This implies that gas exchange that should occur normally via the placenta at this stage must take place outside the uterus in developmentally fetal lungs. Proper alveoli have not yet formed, and the development of the future gas exchange units are still in progress by septation and growth and proliferation of distal respiratory units and capillary networks. Thibeault *et al*. examined the lungs of 30 infants who died of non-pulmonary causes between 22 and 40 weeks' GA and found a particularly large increase in the alveolar–capillary membrane surface area from 22 to 32 weeks' gestation, followed thereafter by a slower increase over the remaining gestation period.[255] Thus, in EP-born infants, gas exchange takes place over significantly less developed alveolar–capillary membranes while the total available surface area accessible for gas exchange is significantly reduced, compared to term-born infants. This means that both the first and second diffusion criteria of Fick's law are affected in the preterm neonate, resulting in impaired gas exchange.

With ongoing respiratory support and medical treatment given to the EP-born infant as part of neonatal intensive care, the development of the alveoli and pulmonary vasculature in the neonate continues to take place, although in a completely different environment, compared to the protective interior of the uterus. Studies have shown that the development of the alveolar–capillary units continues in preterm infants after birth, but at a much slower rate, compared to controls.[227] However, airway obstruction is common in EP survivors,[199, 256] and clinical measurement of pulmonary gas exchange is important for determining the long-term consequences at the alveolar level. Thus,

measurements of lung diffusing capacity parameters in subjects born EP are of great interest in long-term follow-up of this patient group.

Several studies have measured lung diffusing capacity in infants, children, and young adults born EP. Some of this research will be presented in Section 2.1.3.1.

2.1.3.1 Lung diffusing capacity following preterm birth

2.1.3.1.1 Infants and toddlers

Two studies measured DL_{CO} in EP-born infants and toddlers with BPD, compared to term-born controls,[257, 258] by using a technique where the breath-holding manoeuvre involved inducing a respiratory pause of 4 seconds in sleeping subjects.[259] Results from both studies showed reduced DL_{CO} and similar V_A in the BPD group, compared to term-born controls. Chang *et al*. also measured D_M and V_C in infants and toddlers with BPD, compared to healthy term-born controls, and found both parameters were reduced in the BPD group.[258]

2.1.3.1.2 School-aged children

Most studies of preterm-born children aged 7–12 years have reported reduced DL_{CO} and/or K_{CO}, and similar V_A, compared to term-born controls,[260-268] with relatively modest DL_{CO} reduction of approximately 10%. No clear association between reduced DL_{CO} and chronic lung disease of infancy (CLD) and BPD status has been demonstrated, as different studies have described inconsistent results.[261-263, 268]

A Danish study measuring the diffusing capacity of the lung for NO (DL_{NO}) in 11-year-old EP-born children found that D_M, but not V_C, was reduced in the preterm group, indicating a greater relative impairment of the alveolar membrane, compared to the alveolar capillary circulation.[267]

2.1.3.1.3 Adolescents and adults

Most studies investigating diffusing capacity in preterm-born adolescents and young adults have reported reduced DL_{CO} in the preterm group, compared to term-born controls.[269-271] In the Swedish LUNAPRE (Lung Obstruction in Adulthood of Prematurely Born) study, two groups (with and without BPD) of 19-year-old preterm-

born subjects (GA ≤32 weeks) were compared to a group of term-born subjects with asthma and to a group of healthy term-born controls.[271] Both preterm groups had lower DL_{CO}, compared to term-born asthma patients and healthy controls. Narang *et al*.[272] demonstrated reduced DL_{CO} in preterm-born subjects (mean GA of 31.5 weeks) at age 21 years, compared to a group of term-born controls. The same study also measured DL_{CO} during and after exercise and found that DL_{CO} normalized in the preterm group during exercise but was again reduced after a recovery period.[272] A recent French study found no significant differences in DL_{CO} in adolescents born very preterm (GA 22–32 weeks), compared to term-born controls, and also reported similar D_M, but lower V_C, values in the preterm group.[273]

2.1.3.2 Lung diffusing capacity in relation to pulmonary hypertension

As previously mentioned, the development of PH following EP birth and PPROM is complex and is probably related to factors contributing to the causal chain of events leading to preterm birth, to the interruption of pulmonary development, and to treatment given following preterm birth. Preterm-born infants following PPROM are particularly prone to developing PH.[146] Moreover, EP survivors, and especially those with BPD, are likely at increased risk of developing COPD later in life. Patients with COPD are at increased risk of developing PH, and COPD patients with PH have increased morbidity and mortality rates, compared to those with COPD alone.[274-276] In COPD patients with PH, severely impaired diffusing capacity has been associated with higher mortality, and it has been suggested that DL_{CO} may be a prognostic marker in this patient population.[275] Even mild COPD has been found to be associated with significant abnormalities in pulmonary microvascular blood flow, resulting in worsening disease progression.[219] Given prematurity, COPD, and PH seem to be interrelated, this triad represents a worrying scenario in terms of health outcomes later in life.

2.3 Gaps in current knowledge

Lung diffusing capacity measurements are widely used in follow-up studies of children born EP. While there are data showing intra- and inter-session variability in

the SB technique in adult populations,[249, 250, 277] variability data in children are sparse.[251] To our knowledge, there are no published data assessing inter-session variability (reproducibility) of diffusing capacity measurements between EP- and term-born children. Such data would allow proper comparisons of outcomes between groups. Moreover, studies exploring reproducibility of the diffusing capacity method among children (both EP- and term-born), compared to adults, undoubtedly will add new knowledge.

Cross-sectional studies of lung diffusing capacity have provided valuable data on long-term consequences of EP birth. However, longitudinal studies are lacking—these would help expand our knowledge on long-term development of alveolar function following EP birth, particularly in understanding whether lung function impairments in childhood persist into adulthood, and if so, the underlying mechanisms.[278] Recent studies using magnetic resonance imaging (MRI) have indicated that alveolarization continues throughout adolescence and that structural catch-up growth seems to occur in preterm-born individuals.[68, 69] Longitudinal follow-up at different ages would help determine whether there is also alveolar functional catch-up.

Impaired alveolar development could theoretically hamper lung diffusing capacity through reduced surface area that is accessible for gas exchange and/or thickening or impaired development of the alveolar–capillary membrane, or impaired formation of vascular components. Findings of reduced DL_{CO} in EP-born individuals has led us to speculate on possible underlying causes, as there is limited knowledge of which component (membrane versus vascular) is most affected. Thus, studies investigating DL_{CO} by differentiating between its membrane and vascular components would help elucidate the mechanisms underlying reduced gas exchange.

Finally, birth following PPROM implies that fetal lung development occurs in complete absence of, or in the presence of only minimal, amniotic fluid. This phenomenon represents an opportunity for research that will help to further our understanding of lung physiology following EP birth from a different perspective.

3 Study aims and hypotheses

3.1 Study aims

The overall aim of the work presented in this thesis was to investigate alveolar function in childhood, adolescence, and early adulthood in individuals who survived EP birth and those who survived PV-PPROM, with additional comprehensive cardiopulmonary investigations in the latter group.

The specific aims of the study, as described in the three research papers presented in this thesis, are detailed below:

Study aim 1: Lung diffusing capacity measurements – methodological comparison (Paper I):

Study aim 1(i): Reproducibility of measurements using the SB technique in children

- To compare reproducibility of lung diffusing capacity measurements between children and adolescents born EP and term-born controls.

Study aim 1(ii): Comparison between the SB and IB methods of measurement

- To compare the SB and IB methods in relation to lung diffusing capacity measurements in children and adolescents born EP and term-born controls.

Study aim 2: Comparison of lung diffusing capacity between subjects born EP and term-born controls (Papers I and III):

- To investigate whether pulmonary gas exchange, as demonstrated by lung diffusing capacity measurements, is reduced in EP-born children, adolescents, and young adults, compared to term-born controls.

Study aim 3: Longitudinal development of K_{CO} and DL_{CO} and its subcomponents from mid-childhood to adulthood (Paper III):

- To compare long-term development of lung diffusing capacity and its subcomponents membrane diffusion (D_M) and pulmonary capillary blood volume (V_C) between subjects born EP and controls born at term.

Study aim 4: Cardiopulmonary outcomes in children surviving PV-PPROM (Paper II):

- To examine cardiorespiratory outcomes, including lung diffusing capacity, in children who survived PV-PPROM before 22 weeks' GA, in comparison with individually matched controls born EP but without PV-PPROM.

3.2 Study hypotheses

Hypothesis 1, $H0_1$: There is no difference in reproducibility of measurements of lung diffusing capacity between EP-born and term-born subjects.

Hypothesis 2, $H0_2$: There are no differences in parameters of lung diffusing capacity obtained with the SB technique compared to the IB method.

Hypothesis 3, $H0_3$: There are no differences in lung diffusing capacity measurements between children, adolescents, and young adults born EP versus control subjects born at term.

Hypothesis 4, $H0_4$: There are no differences in development of lung diffusing capacity and its subcomponents between EP-born subjects and term-born controls, from mid-childhood to adolescence and from adolescence to young adulthood.

Hypothesis 5, $H0_5$: There are no differences in lung diffusing capacity and cardiopulmonary outcomes between children born EP with PV-PPROM versus matched preterm-born control subjects with no PV-PPROM.

4 Study design, subjects, and methodology

4.1 Study design

4.1.1 The EP studies (Papers I and III)

The EP studies were comprehensive population-based, longitudinal, controlled follow-up studies involving two EP-born subject cohorts (born in 1982–5 and 1991–2). Participants were assessed on two occasions: (1) first assessment in 2001–2 at age 18 and 11 years, respectively; and (2) second assessment in 2008–9 at age 25 and 18 years, respectively. The two cohorts included subjects who survived EP birth (preterm-born) and those who were term-born (controls). The preterm-born cohorts were identified retrospectively from neonatal protocols at Haukeland University Hospital (HUS).[279] Data from the antenatal, prenatal, and neonatal periods were recorded before outcomes were assessed.

4.1.2 The PPROM study (Paper II)

The PPROM study was a retrospective, population-based, cross-sectional follow-up study. Participants were identified retrospectively from hospital databases, and all prenatal and neonatal data were recorded before outcome assessments.

4.2 Study subjects

Two population-based cohorts of EP-born subjects were individually matched to randomly selected term-born controls. In addition, a cohort of premature children born after PPROM before 22 weeks' GA was gender-matched to premature controls born at similar GA, but without PPROM.

4.2.1 Extremely preterm-born subjects (Papers I and III)

All long-term survivors who were born at ≤28 weeks' GA or with BW ≤1000 g to mothers living within a defined area (Hordaland and Sogn og Fjordane) covered by the Western Norway Regional Health Authority in the periods of February 1991 to June 1992 and January 1982 to December 1985 were invited to participate. HUS is the only regional institution admitting and caring for EP-born infants, serving a population of

approximately 500 000. The annual birth rate at the time of the study was approximately 6700 children. Seventy-six of the 81 subjects (94%) were in-born at HUS. At the first assessment in 2001, there were 51 eligible surviving subjects from the first cohort and 35 from the second.

Determination of GA was primarily based on the mother's LMP. Ultrasound scanning, systematically available only to the 1991–2 cohort, was performed before the 21st week of pregnancy. If delivery dates from the LMP and ultrasound scanning differed by >2 weeks, preference was given to the date obtained by ultrasound scanning. If delivery dates differed by >3 weeks, paediatric postnatal assessment was undertaken.[280] In cases of doubt, an uninvolved senior obstetrician was consulted before decisions were made.

Subjects admitted to the NICU at HUS were eligible for study participation. The majority of the senior staff involved were the same people in both inclusion periods. Neonatal data were obtained from hospital charts, mostly in a tabulated format. Other background data were retrieved from questionnaires and medical charts at the time of study assessments. The decision to wean from O_2 was based primarily on transcutaneous measurements for the 1982–5 birth cohort and on oximetry for the 1991–2 birth cohort.

4.2.2 Term-born control subjects (Papers I and III)

For each preterm-born participant, the temporally nearest term-born child (GA ≥37 weeks) of the same sex with a BW of between 3 and 4 kg (Norwegian 10th to 90th percentile) was recruited at the time of the first follow-up, using hospital birth records as data source. Matching term-born controls were recruited through a letter of invitation for study participation sent to their parents. If a subject declined the invitation, the next subject was approached and so on, until a 1:1 control group had been recruited. The purpose of the control group was to reflect the preterm population with respect to relevant attributes, except for GA at birth. Hence, there were no exclusion criteria, except expected inability to perform in assessments. Also, the aim was to keep the travel time from participants' home address to the hospital under 1 hour, for practical and financial reasons.

4.2.3 Subjects in the PPROM study (Paper II)

This was a retrospective study of children born alive in 2000-2004 and admitted to the NICU at HUS after PPROM before 22 weeks' GA and with a minimum latency period of 14 days. Eligible subjects were identified from hospital databases and invited to participate if still alive. The diagnosis of PPROM was based on subjective complaints of vaginal amniotic fluid discharge, a confirmative gynaecological examination, and low amniotic fluid volume on ultrasonography.[281] One individually matched control subject without PPROM <24 weeks' GA was recruited for each case by identifying the next-born child of the same sex and GA from hospital birth protocols. If that subject declined participation, the next-born subject was approached, and so on.

Of 17 eligible children, four (24%) had died, all during neonatal intensive care, and two declined to participate. Eleven subjects in the PPROM group were therefore examined, along with the same number of individually matched preterm-born controls.

4.3 Study methodology (measurements and testing conditions)

4.3.1 The EP studies (Papers I and III)

Subjects in the EP studies were examined in 2001–2 and subsequently in 2008–9 at the cardiopulmonary laboratory of the Paediatric Department at HUS. For both examination sessions, all subjects were assessed on two different days within a 2-week period. A full medical assessment was performed by one of two paediatricians in 2001–2, and one of the two performed the majority of these assessments also in 2008–9, while a third paediatrician carried out the remaining assessments. Anthropometric measurements (height and weight) were performed, along with skin prick tests (SPTs), blood sampling (including for general haematology measurements and specific serum immunoglobulin E (IgE) assays), and morning urine samples. The same respiratory physiologist performed all pulmonary function and exercise testing using similar equipment on both visits. The manufacturer's representative in Norway carried out, upon request, annual software, and hardware checks on the testing equipment in the cardiopulmonary laboratory.

If a test subject had respiratory symptoms that could be related to either an asthma exacerbation or a viral infection in the 2 weeks prior to testing, their assessment was rescheduled. To avoid any influence on pulmonary function test results, study participants were asked to stop using inhaled corticosteroids, long-acting β2-agonists, and oral leukotriene blockers 1 day prior to testing, and to avoid use of short-acting β2-agonists unless necessary.[282] Participants were also asked not to drink caffeine-containing drinks 4 hours prior to testing,[283] and to refrain from cigarette smoking on the test day. Subjects were also requested not to use antihistamines, if possible, in the week prior to testing.

Participants and/or their parents were given three different sets of questionnaires to complete. The Child Health Questionnaire (CHQ)[284] was used to assess quality of life, while the International Study of Asthma and Allergies in Childhood (ISAAC)[285] questionnaire mapped respiratory symptoms. Data from these two questionnaires were not considered in the work presented in this thesis. Additional questionnaires were designed to map the remaining relevant demographic variables and other health-related information (see Appendix I).

4.3.2 The PPROM study (Paper II)

The PPROM study was carried out from August 2012 to October 2015. Data on demographic characteristics, current and previous diagnoses, and neurodevelopmental clinical course were obtained from a parental questionnaire (see Appendix II), as well as from medical records obtained from relevant hospitals and county public health care institutions where all the children were followed up routinely. Thus, data on neurodevelopmental outcomes were not clinically assessed as part of the study presented in Paper II, but only reported based on professional assessment from regular medical follow-up provided by community health services. Blindness, deafness, or quadriplegic cerebral palsy were classified as major neurological disabilities. Gross and fine motor delays were defined as such if reported by hospital or community physiotherapists. Clumsiness was reported by participants' parents, but only defined as such if the child had physiotherapy for this reason at some stage during preschool or school years. Psychiatric disorders were defined as such based on reports from

community or hospital child psychiatrists/psychologists. Learning disabilities and/or difficulties with concentration, attention, or interaction were defined based on reports from child psychologists or special education experts and recorded as such in this study if the child had received educational assistance at school. The physician recorded relevant details on medical history and medical examination findings in a protocol used for all participants (see Appendix III). Respiratory symptoms and a diagnosis of asthma were ascertained using the ISAAC questionnaire.[285]

4.3.3 Definitions

Mild and moderate/severe BPD were defined as requirement for supplemental O_2 at ≥28 postnatal days and at GA ≥36 weeks, respectively.[102] For the PPROM study, BPD was defined as requirement for assisted ventilation or O_2 supplementation at 36 weeks' GA. Relevant information on the above was obtained from standardized nurse diagrams which are kept as part of hospital charts.

Maternal smoking in pregnancy was defined as daily or occasional smoking by the participants' mother, as self-reported during pregnancy.

4.3.4 Pulmonary function tests

The same pulmonary technician performed all pulmonary function tests for all studies. The testing equipment and devices were calibrated regularly following the manufacturer's instructions.

The main focus of the studies presented in this thesis was on alveolar function in individuals born preterm. Therefore, the methods for measuring lung diffusing capacity will be described in detail, with brief descriptions given on other pulmonary function tests. Participants in the PPROM study were also assessed by echocardiography and cardiopulmonary exercise testing, which will be described only briefly.

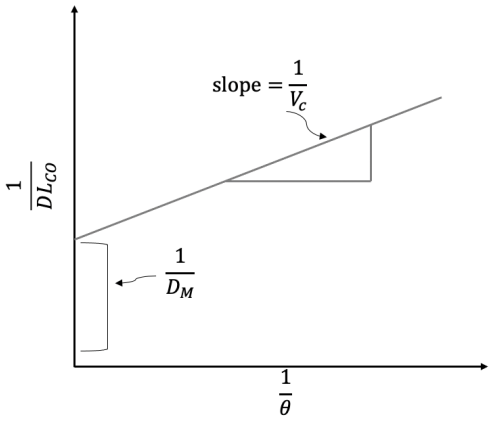
4.3.4.1 The single-breath method for measuring lung diffusing capacity

Measurements of single-breath DL_{CO} were done with Vmax equipment (SensorMedics, Yorba Linda, CA, USA) in agreement with ERS standardized

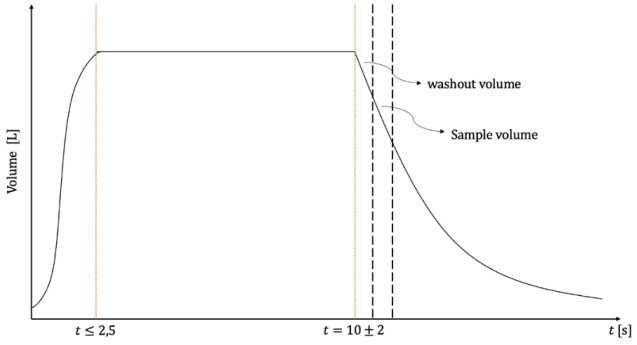
criteria.[230, 286] DL_{CO}, V_A, K_{CO}, D_M, and V_C values were recorded. D_M and V_C values were obtained by measuring DL_{CO} at two different O_2 tensions (21% and 80%). In the 2001–2 EP studies, SB-DL_{CO} was measured on two test days, with the additional test for D_M and V_C randomized to take place on either the first or second test day. In the 2008–9 EP studies, as well as in the PPROM study, SB-DL_{CO}, D_M, and V_C were measured only once. Data were reported as raw data (Papers I and II) and as *z*-scores (Paper III). *z*-scores were calculated using the Stanojevic GLI-2017 regression equations for Caucasians, corrected for age, height, and sex.[247] D_M and V_C were determined using the Roughton–Forster equation.[223]

4.3.4.1.1 Practical performance

The test subject was seated, with a nose clip in place. A few tidal breaths were recorded to establish a stable end-expiratory baseline. Subjects were then asked to exhale to RV within a limit of 6 seconds (3 seconds for children), at which point the gas supply switched to the test gas, containing a mixture of 0.3% CO, 0.3% CH_4, and 21% (or 80%) O_2, balanced with nitrogen. Subjects then inhaled rapidly to TLC. Inspiration should be achieved in ≤2.5 seconds. After 10 ± 2 seconds of breath-holding (while performing neither Valsalva nor Müller manoeuvre), subjects exhaled smoothly with no hesitation or interruption. The breath-holding time was calculated using the Jones–Meade method.[287] A mid-expiratory sample of alveolar gas was collected and analysed, discarding the initial volume to clear the anatomical and mechanical dead space (V_D), as contamination of the alveolar sample with dead space gas will cause an underestimation of true CO uptake. This alveolar sample of gas (minus V_D) is therefore used for analysis of CO and tracer gas concentrations. A sample volume (V_S) of 0.50–1.00 L should be collected for analysis. In patients with a vital capacity (VC) of <1 L, a V_S of <0.50 L may be used, provided the V_D has been cleared. CH_4 was the inert gas used to estimate alveolar lung volume (V_A).



By measuring DL_{CO} at two different O_2 tensions, θ (the reaction rate between O_2 and Hb) is variable, and $1/DL_{CO}$ is plotted against $1/\theta$ at the different inspired O_2 pressure levels. The slope of this relationship is $1/V_C$ and the intercept is $1/D_M$.



The criteria for an acceptable manoeuvre were: inspiratory volume of at least 90% of VC, inspiratory time ≤2.5 seconds, and breath-holding time of 10 ± 2 seconds. When two acceptable manoeuvres were obtained with values no more than 10% apart, the mean value was recorded for further analysis. A maximum of four attempts at intervals of a minimum of 5 minutes were allowed. According to the 2005 ERS/ATS[230] recommendations, at least 4 minutes must be allowed between manoeuvres to allow for adequate elimination of the test gas from the lungs. If only one acceptable manoeuvre was obtained, that value was recorded. In a few cases, data

with adequate curves within time limits and with inspiratory volumes below, but close to, 90% of VC were accepted.

4.3.4.2 The intra-breath method for measuring lung diffusing capacity

The Vmax equipment (SensorMedics) was used for the intra-breath measurements. The IB technique was based on measurement taken throughout a single expiration. After calibration, the test subject had a mouthpiece and nose clip secured in place, before initiating stable tidal breathing. The subject was thereafter instructed to exhale completely to RV, at which point the mouthpiece was switched to the test gas containing 0.3% CO, 0.3% CH_4, 0.3% acetylene (C_2H_2), and 21% O_2, balanced with nitrogen. The subject then fully inhaled to TLC and, without breath-holding at TLC, exhaled slowly and smoothly at a targeted flow rate of 0.3–0.6 $L \cdot s^{-1}$ until the test is ended by the computer. The concentration of the test gases was measured during exhalation, using the collection interval during the alveolar gas plateau, i.e. after washout of all dead space gas and before the closing volume gas. This interval was typically between 20% and 80% of the expired VC. For an acceptable manoeuvre, the inspiratory volume should be ≥90% of the VC and the inspiratory time ≤2.5 seconds. The same rules as for SB-DL_{CO} were applied for reproducibility,[230] and the same variables were recorded.

4.3.4.3 Spirometry

Spirometry measurements were performed according to ERS/ATS standardization,[288-290] with use of a SensorMedics Vmax equipment (SensorMedics, Yorba Linda, CA, USA). FVC, peak expiratory flow (PEF), FEV_1, and forced expiratory flow at 25%, 50%, and 75% of FVC ($FEF_{25–75}$) were recorded. The results were expressed as *z*-scores and percentages of predicted values using the GLI all-age reference equations.[71, 291]

The test subject initiated stable tidal breathing and thereafter performed a full inspiratory manoeuvre followed by a forced expiratory manoeuvre. The manoeuvre was repeated until three acceptable curves were obtained. The highest values for FEV_1 and FVC obtained from technically acceptable flow–volume loops were recorded,

whereas $FEF_{25–75}$ was obtained from the flow–volume loop with the highest added value of FEV_1 and FVC.

4.3.4.4 Body plethysmography

Static lung volumes were determined with use of an Autobox 6200 plethysmograph (SensorMedics). Standard ERS criteria were applied for measurements and calibration.[289, 292] Inspiratory (IRV) and expiratory reserve volume (ERV), FVC, RV, and TLC were recorded, and airway resistance against tidal respiration (R_{aw}) was measured. The panting technique was used to obtain pressure–volume loops used to estimate the thoracic gas volume (VTG), from which functional residual capacity (FRC) was calculated. Lung volumes were recorded as percentage of predicted values, ratios, or *z*-scores.[239, 293] Reproducibility of lung volume measurements was demonstrated for the EP-born cohorts, as published previously.[294]

4.3.5 Cardiopulmonary exercise testing

Cardiopulmonary exercise capacity was measured with use of an incremental treadmill (Woodway ELG 70, Woodway, Weil am Rhein, Germany) test, according to a modified computerized Bruce protocol,[295] where speed and incline were gradually increased every 90 seconds starting from an initial slow-walking phase. The test was stopped when the subject reported or showed exhaustion, preferably corroborated by a plateau in peak oxygen uptake (peak VO_2) or heart rate response.[296, 297] Variables of gas exchange were measured breath-by-breath using a face mask connected to a Vmax29 cardiopulmonary exercise unit (SensorMedics). Peak VO_2 was reported as raw data unadjusted and adjusted for body weight, and as percentages of predicted values. The reference peak VO_2 values were derived from cycle ergometer testing due to lack of adequate treadmill data for children at the time of the study in this group.[298]

Exercise data have previously been published for the EP cohorts [299-301], and only data from the PPROM study as described in Paper II is reported in this thesis.

4.3.6 Echocardiography

Echocardiography assessments were conducted in the PPROM study with use of a digital Vivid 7 ultrasound scanner (GE Vingmed, Horten, Norway). Two paediatric cardiologists performed all assessments, which focused mainly on right ventricular function and indirect markers of pulmonary arterial pressure. Conventional greyscale cine-loops and pulsed and continuous wave Doppler recordings of blood flow velocities were sampled. Right ventricular performance was measured in centimetres by tricuspid annular plane systolic excursion (TAPSE) in M-mode. The tricuspid pressure gradient was calculated from each recording according to the Bernoulli equation, using maximal tricuspid regurgitation jet velocity (TRJV). Data were standardized for body surface area.[302] All echocardiographic assessments were performed before exercise testing.

4.4 Statistics

Statistical analyses were performed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY, USA), versions 19 (Paper I), 22 (Paper II), and 25 (Paper III), and R version 4.0.2.[303] The mixed-effects models presented in Paper III were fitted with the R package 'lme4' version 1.1-23.[304] The Bland–Altman plots presented in Paper I were created with MedCalc version 12.0 (MedCalc Software, Mariakerke, Belgium).

The statistical power of the long-term EP studies was determined by the available number of participants from the first assessment. The original project was designed in 2001 to evaluate a series of outcomes, with variables with different and partly unknown distributions. By nature, a priori sample size calculation is complex in this setting. The sample size was calculated to detect a clinically relevant decrease in the EP-born groups for the main outcome measure of the overall study, which was FEV_1. The study was originally designed in 2001 to have 90% power to detect differences exceeding 7.5 percentage points in FEV_1 between the extremely preterm-born and term-born groups, with a two-sided significance level of 0.05. Splitting the data set by BPD or by gender would, by nature, reduce the number of subjects in the groups and therefore also reduce the statistical power of subgroup analyses.

For the PPROM study, 11 eligible children were identified, implying that the study had 80% power to detect a group difference for FEV_1 of approximately 12–15%, providing SDs in line with those of the EP-born groups who had been examined previously, and a two-sided significance level of 0.05.

Data were presented as group means or medians with 95% CI or ranges, or as numbers with proportions (%), as appropriate. Due to the design of the studies with individually matched subjects and controls, statistical analyses appropriate for the paired design were used whenever possible. Differences between groups (i.e. between EP-born and term-born subjects within and between cohorts) were assessed with Welch's one-way ANOVA/*t*-tests for continuous variables, and with Pearson's chi-squared test and McNemar's test for categorical variables. The mixed linear model (MLM) was used for some of the paired group comparison analyses. Linear regression analyses were used to test for associations between perinatal exposures and lung diffusing capacity. Reproducibility of lung diffusing capacity measurements in Paper I was determined by calculating SDs of the differences between measurements obtained on the two different examination days. The SDs were used to calculate the CVs (expressed as 1 SD as a percentage of the average value of the two measurements) and to determine the 95% limits of agreement.[305, 306]

Longitudinal mixed effects models were used for longitudinal analyses in the EP studies to estimate mean values and differences in mean values for *z*-DL_{CO}, DL_{CO} %-predicted, *z*-V_A, *z*-K_{CO}, K_{CO} %-predicted, D_M, and V_C at each time point. The mixed effects models allow inclusion of all participants, including those with incomplete follow-up data. The models therefore reduce bias caused by missing data and increase the precision of estimates.[307] The explanatory variables were cohort, age (categorical), and subject category (i.e. term-born versus EP-born). Grade of BPD severity was also used as an explanatory variable for analyses presented in the supplementary table in Paper III. All interactions were included to make the models maximally flexible, and subjects were included as a random effect. Residual plots were examined, and any errors in the original data corrected. To examine if development for EP-born subjects tracked similarly with that for term-born subjects, simplified models with parallel lines were fitted for the two groups (but possibly with different slopes for

the two cohorts) and compared with the fully flexible models using likelihood ratio tests.

5 Ethics

The Regional Committee on Medical Ethics of the Western Norway Health Authority approved all studies presented in this thesis (REK-Vest 99.2000, 240.07, and 2010-3052). All participants and/or their parents gave informed written consent to participate, as required by Norwegian law. All subjects were informed of their right to withdraw from participation at any time during the study. Except venepuncture for the purpose of blood sampling, none of the study tests performed were considered particularly unpleasant or represented any danger to participants. Those who were uncomfortable with having their blood samples taken were offered local anaesthetic plasters (Emla®) to minimize pain associated with the procedure.

Extensive testing of individuals who considered themselves healthy always represents a risk of detecting unexpected pathology or raising issues or questions that might be difficult to predict in advance. The hospital's well-established close interdepartmental links, as well as the ease with which such circumstances could be discussed with expert colleagues, along with the possibility of referral to specialized care if considered necessary, mitigated this problem. Moreover, appropriate, and sound advice to participants and their parents, as well as participants' general practitioners, could also be provided.

EP birth implies the need of advanced and complex treatment, often lengthy, for the newborn, and the burden of subsequent illness is sometimes high. Having regular follow-up examinations throughout childhood may promote a sense of security in both participants and their parents and at the same time may set up the right arena for discussions or voicing frustrations. On the other hand, repeatedly focusing on symptoms and functional impairments can lead to unnecessary feelings of illness or of being different from one's peers. The overall impression here was that the majority of preterm-born subjects appreciated these meeting points provided by the research teams. All test results were also openly discussed with the participants and their guardians.

6 Summary of results

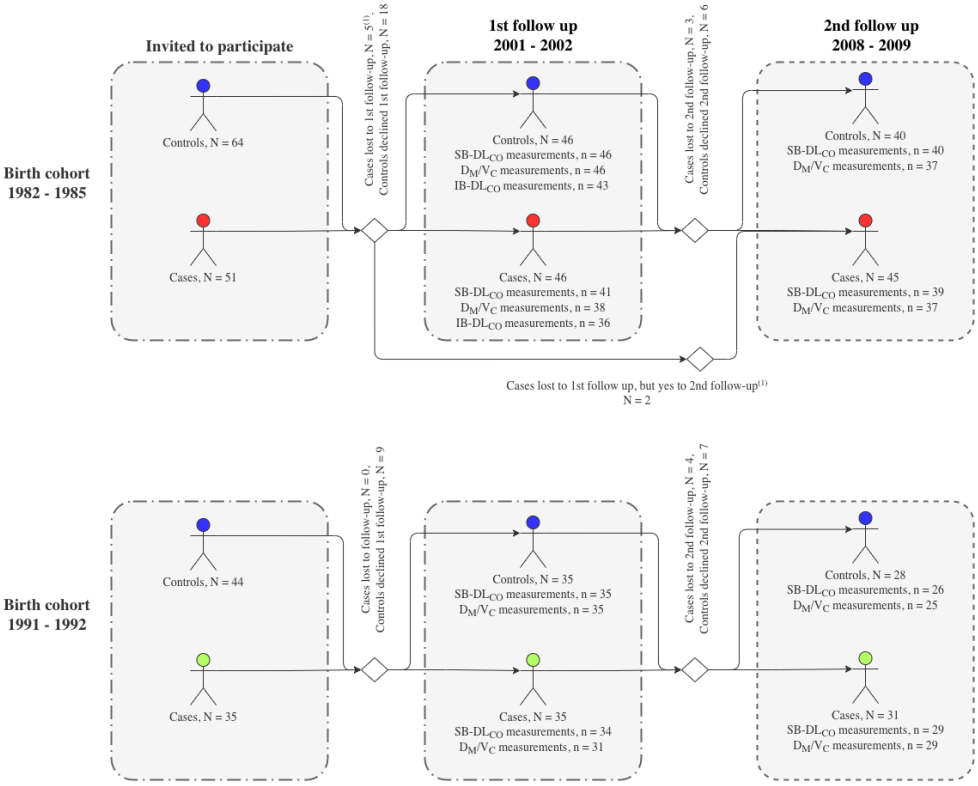
This section presents results from Paper I, II, and III that constitute the basis for the research described in this thesis. Some supplementary data (unpublished data) are also presented with the intention to add clarification to the reported results.

6.1 Study cohorts (Papers I and III)

In the EP-born cohorts, 83 (96.5%) of a total of 86 eligible survivors participated at least once, including 48 and 35 subjects from the 1982–5 and 1991–2 birth cohorts, respectively (for details, see Figure 2; based on Figure 1 from Paper III). NICU mortality was 39% and 26% in the 1982–5 and 1991–2 birth cohorts, respectively (p = 0.157).[308]

When recruiting term-born controls, one potential subject was invited for each of the 81 consenting EP-born subjects at the first examination session in 2001–2, and of those invited, 61 (75%) responded positively. On average, 1.3 term-born individuals had to be approached for every EP-born participant to establish the complete 1:1 matched control group. In the 1982–5 birth cohort, 40 (87%) of the 46 control subjects who participated in the first examination session also attended the second. Corresponding figures for the 1991–2 birth cohort were 28 of 35 (80%).A total of seven participants in both birth cohorts were included based on the BW criterion only (i.e. BW ≤1000 g): two in the 1991–2 cohort (GA 30 and 31 weeks) and five in the 1982–5 birth cohort (mean GA 30 weeks). All subjects in the 1982–5 birth cohort and all but two subjects in the 1991–2 birth cohort were Caucasian.

***Figure 2**: Recruitment process and participation in diffusing capacity measurements for the EP studies*



(1) Cases loss to first follow-up: Two subjects were inaccessible (one had moved abroad, and the second subject never responded to the invitation), one was excluded due to severe Eisenmenger syndrome, and two declined to participate during the 2001-2 measurements.

6.2 Haemoglobin concentrations (Papers I and III)

Hb concentrations were within normal ranges for most participants in both birth cohorts (unpublished data) and are presented in Table 1.

Table 1. Haemoglobin levels for both birth cohorts at both examinations.

		First examination 2001-2001			Second examination 2008-2009		
		EP n= 43* and 34**	Term-born n= 44* and 32**	p-value	EP n= 37* and 20**	Term-born n= 27* and 15**	p-value
1982-1985 cohort	Female	12.7 (12.4–13.1)	12.8 (12.4–13.1)	0.95	13.7 (13.3–14.1)	13.4 (12.5–14.2)	0.39
	Male	15.0 (14.5–15.4)	14.7 (14.3–15.0)	0.27	16.2 (15.7–16.7)	15.1 (14.5–15.6)	0.004
1991-1992 cohort	Female	13.0 (12.6–13.4)	12.7 (12.5–13.0)	0.63	13.4 (12.8–14.1)	13.3 (12.8–13.7)	0.62
	Male	13.0 (12.4–13.6)	12.9 (12.5–13.2)	0.32	15.4 (14.7–16.1)	15.4 (14.0–16.8)	0.99

Haemoglobin levels measured by venous punctures in the participants in the 1982-1985 and 1991-1992 birth cohorts. EP= extremely preterm. *In the 1982-1985 birth cohort. **In the 1991-1992 birth cohort.

6.3 Study aims 1 and 2: Reproducibility of SB-DL_{CO}, comparison between the SB and IB techniques, and comparison of DL_{CO} following EP birth versus term birth (Paper I)

SB-DL_{CO} measurements were performed on both test days, with the aim to explore inter-session variability of the SB technique, as well as comparing DL_{CO} measurements and variability between the EP-born and term-born groups. DL_{CO} measurements were also carried out in the 1982–5 birth cohort with the IB technique, to facilitate comparison between the two techniques.

6.3.1 Study aim 1(i): Reproducibility of SB-DL_{CO} measurements using the SB technique in children (Paper I)

Inter-session CV for SB-DL_{CO} measurements was around 8% for children aged 10 years in the 1991–2 birth cohort, which was similar to a CV of 7.5% observed in term-born young adults in the 1982–5 birth cohort. Inter-session differences in percentage

of absolute numbers revealed day-to-day measurement variability of around 5% for 10-year-old children in the 1991–2 birth cohort, compared to around 3.5% for young adult control subjects in the 1982–5 birth cohort. The CV for K_{CO} varied from 7.0% to 8.4%.

The EP-born groups had slightly higher CVs, compared to control groups (9.6% versus 7.5%, respectively, in the 1982–5 birth cohort, and 8.2% versus 7.7%, respectively, in the 1991–2 cohort), indicating marginally higher variability among EP-born subjects. The same trends were observed for the 95% limits of agreement, expressed as ± 1.96 SD of the average difference between replicate measurements (taken on days 1 and 2). The highest variability was seen for EP-born subjects in the 1982–5 birth cohort. The 95% limits of agreement varied from 13.8% to 16.4% for K_{CO}.

Values of SB-DL_{CO} and SB-K_{CO} obtained on day 1 exceeded those obtained on day 2, varying from a mean of 0% to 5.4%, with the greatest differences seen in the 10-year-olds.

6.3.2 Study aim 1(ii): Comparison between the SB and IB methods of measurement (Paper I)

Measurement of lung diffusing capacity using both the SB and IB techniques was performed only in the 1982–5 birth cohort. Measurements obtained with the SB technique exceeded those with the IB technique. Mean ± SD differences between SB-DL_{CO} and IB-DL_{CO} were 0.48 ± 0.96 $mmol \cdot min^{-1} \cdot kPa^{-1}$ in the preterm group and 0.32 ± 0.83 $mmol \cdot min^{-1} \cdot kPa^{-1}$ in the term-born control group. Alveolar volume measured with the SB method was significantly higher, compared to that measured with the IB method. There was no significant difference in K_{CO} measurements obtained with either method in both preterm and control groups. The 95% limits of agreement for K_{CO} measured with the two techniques were 21.3% for the EP-born group and 17.6% for term-born controls.

6.3.3 Study aim 2: Comparison of lung diffusing capacity between subjects born EP and term-born controls (at the first examination session, 2001–2) (Paper I)

SB-K_{CO} and SB-DL_{CO} were significantly reduced in subjects born EP, compared to term-born controls. Alveolar volume was reduced in EP-born subjects from the 1991–2 birth cohort, compared to term-born controls, but not in those from the 1982–5 birth cohort. Similar findings were obtained with the IB technique where K_{CO} and DL_{CO}, but not $V_{A,}$ were significantly reduced in EP-born subjects, compared to term-born controls.

On average, DL_{CO} was reduced by 12–18%, and K_{CO} by around 7–10%, in EP-born subjects, compared to term-born controls. There was no trend towards improvement or deterioration in lung diffusing capacity in EP-born subjects from the 1991–2 birth cohort, compared to their EP-born peers from the 1982–5 birth cohort. Within the groups born EP, there was no clear association between neonatal BPD severity and lung diffusing capacity measured at follow-up.

6.4 Study aim 3: Longitudinal development of K_{CO} and DL_{CO} and its subcomponents from mid-childhood to adulthood (Paper III)

6.4.1 Longitudinal development of DL_{CO}, V_A, and K_{CO}

The number of successful tests of SB-DL_{CO}, V_A, and K_{CO} are shown in Figure 2. After standardization for age, sex, and height,[248] K_{CO} and DL_{CO} were significantly reduced in all EP-born groups, compared to matched term-born control groups, and remained reduced throughout puberty and early adulthood, with deficits of 0.5 *z*-score or more. Raw data for DL_{CO} measurements showed deficits of around 10% in both birth cohorts, with the largest difference seen in the 10-year-olds (18.5%). V_A did not differ significantly between EP- and term-born groups.

For both EP-born birth cohorts, *z*-DL_{CO}, *z*-K_{CO}, and *z*-V_A developed in parallel with their respective term-born control cohorts over the age span covered by the study, i.e. from age 18 to 25 years in the 1982–5 birth cohort and from age 10 to 18 years in

the 1991–2 birth cohort. The *p*-values for overall tests for a lack of parallelism between EP-born and term-born cohorts from each of the two decades were 0.99, 0.65, and 0.71 for *z*-DL_{CO}, *z*-V_A, and *z*-K_{CO}, respectively. This indicates that development between the two examinations did not differ between the preterm and term-born groups for any of the measured variables.

The reference equations used to calculate *z*-scores (GLI 2017)[247] for DL_{CO}, V_A, and K_{CO} fitted the control population relatively well. Although the mean *z*-score was not zero for most measurements, zero was included in the 95% CIs for the control groups, with two exceptions: V_A for the control group from the 1991–2 birth cohort at first follow-up (mean −0.4, 95% CI −0.7 to −0.1), and K_{CO} for the control group from the 1982–5 birth cohort at first follow-up (mean −0.4, 95% CI −0.7 to −0.1).

6.4.2 Longitudinal development of D_M and V_C

The numbers of successful tests of D_M and V_C are shown in Figure 2. Mean D_M was numerically lower in all EP-born cohorts, compared to term-born cohorts, but significantly so only in the 1991–2 birth cohort at age 10 years. V_C deficits, although smaller, did not reach statistical significance for any of the EP-born cohorts.

Development of D_M *and* V_C in EP-born cohorts was parallel with their respective term-born control cohorts over the age span covered by the study (*p*-values for lack of parallelism were 0.94 and 0.44 for D_M and V_C, respectively).

6.4.3 Lung diffusing capacity in relation to BPD severity

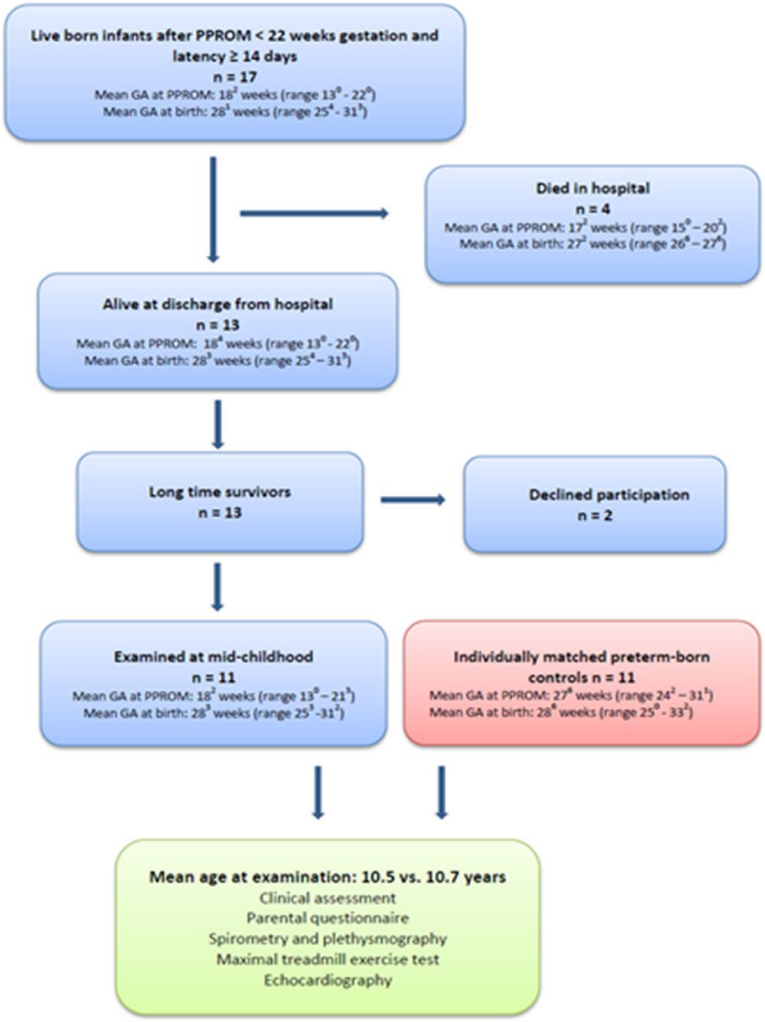
Subgroup analysis based on neonatal BPD status revealed no significant effect on *z*-DL_{CO}, *z*-K_{CO}, and *z*-V_A at follow-up (all *p*-values ≥0.13). However, there was a numerical tendency for higher mean *z*-scores in the moderate/severe BPD group, compared to the group with no or mild BPD.

6.5 Study aim 4: Cardiopulmonary outcomes in children surviving PV-PPROM (Paper II)

6.5.1 Study subjects

The recruitment process of study participants is presented in Figure 3. Eleven children surviving PV-PPROM who fulfilled the inclusion criteria and a group of 11 controls born similarly preterm but without PPROM <24 weeks' GA were examined at a mean age of 10.5 and 10.7 years, respectively. Rupture of membranes in the PV-PPROM group occurred at a mean GA of 18 weeks and 2 days (18^2) (range 13^0 to 21^3 week's GA).

Figure 3. *Recruitment process of the PPPROM study*



The recruitment process of study participants, all born at Haukeland University Hospital in Bergen, Norway in the period 2000–2004.GA, gestational age; PROM, preterm premature rupture of membranes. Reprinted from Journal of Perinatology, 2017 Sep;37(9): 1053-1059, MH Bentsen et al, Mid-childhood outcomes after pre-viable preterm premature rupture of membranes, with permission from Springer Nature.

6.5.2 Pulmonary function tests, echocardiography, and exercise capacity testing

Pulmonary function tests showed that the PV-PPROM group had significantly lower *z*-scores for FEV_1, FEV/FVC, and $FEF_{25–75}$, with a tendency towards more subjects in the PV-PPROM group having respiratory symptoms and using asthma medications, compared to the control group. There was no significant difference in mean percentage predicted DL_{CO} and K_{CO} (raw data) between the two study groups. Raw data for DL_{CO} were not presented in Paper II, and *z*-scores could not be derived as the GLI equations were released in the same year when Paper II was published (2017). To better evaluate alveolar function, detailed lung diffusing capacity data were gathered from the study, including raw data, *z*-scores calculated using the GLI 2017 reference equations,[247] and data for D_M and V_C (unpublished data) (Table 2). There was no significant difference between the groups for any of these variables.

Echocardiography revealed mild tricuspid regurgitation (TR) in all study participants, with a significantly higher TR velocity in the PV-PPROM group, compared to controls, which indicates mild PH in the PV-PPROM group. Mean tricuspid pressure gradient was higher in the PV-PPROM group, compared to the control group, whereas right ventricular function and dimensions were normal and similar in both groups.

Exercise capacity testing showed significantly lower peak VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$) in the PV-PPROM group, compared to the preterm-born control group. However, values were within the normal range, as defined by the applied reference equations.

Table 2. Diffusing capacity variables for subjects born after PV-PPROM and controls matched for GA, age, and gender.

	PV-PPROM	Control	p-value
DL_{CO}	4.2 (3.0 to 5.3)	5.0 (3.9 to 6.1)	0.26
V_A	2.8 (2.0 to 3.6)	3.1 (2.4 to 3.8)	0.55
K_{CO}	1.5 (1.2 to 1.7)	1.7 (1.4 to 1.9)	0.21
***z*-DL_{CO}**	–1.3 (–2.2 to –0.4)	–0.8 (–1.5 to –0.07)	0.34
***z*-V_A**	–0.5 (–1.8 to 0.8)	–0.3 (–0.8 to 0.09)	0.77
***z*-K_{CO}**	–0.9 (–1.7 to –0.2)	-0.5 (–1.2 to 0.2)	0.38
D_M	6.5 (4.0 to 9.0)	7.9 (4.9 to 10.9)	0.42
V_C	50.7 (37.6 to 63.8)	52.8 (40.0 to 65.5)	0.79

Abbreviations: PV-PPROM: Pre viable premature preterm rupture of membranes; GA: gestational age; DL_{CO}: Diffusing capacity of the lung for carbon monoxide ($mmol \cdot min^{-1} \cdot kPa^{-1}$); V_A: Alveolar volume (L); K_{CO}: Transfer coefficient of the lung for carbon monoxide ($mmol \cdot min^{-1} \cdot kPa^{-1} \cdot L^{-1}$); D_M: Alveolar-capillary membrane conductance ($mmol \cdot min^{-1} \cdot kPa^{-1}$); V_C: Pulmonary-capillary blood volume (mL). Absolute numbers (95% CI) are reported for DL_{CO}, V_A, K_{CO}, D_M and V_C while DL_{CO}, V_A, K_{CO} also are reported as z-scores (95% CI).

7 Discussion

This study showed that 10-year-old children could perform the single-breath DL_{CO} test with similar reproducibility as young adults, and reproducibility of the technique was also similar in both EP-born and term-born control groups. Compared to the SB technique, there was a tendency for the IB technique to underestimate DL_{CO} and V_A, but not K_{CO}. Compared to matched controls born at term, parameters of lung diffusing capacity were reduced in EP-born subjects from both birth cohorts of 1985–2 and 1991–2. Development of parameters of lung diffusing capacity from age 10 to 25 years in EP-born subjects tracked in parallel with term-born subjects, albeit at lower levels, with no signs of pubertal catch-up growth or early onset of decline at age 25 years. Further, the study showed that the membrane component of DL_{CO} was numerically reduced at both time points in both EP-born birth cohorts, although significantly so only at the first examination in the younger birth cohort. Data for V_C did not show similar deficits and increased in parallel in both EP-born and term-born cohorts, also from age 18 to 25 years. D_M increased in EP-born cohorts in parallel with term-born cohorts from age 10 to 18 years and remained stable from age 18 to 25 years.

EP-born subjects exposed to PV-PPROM had more severe airway obstruction and poorer exercise capacity ($mL \cdot kg^{-1} \cdot min^{-1}$), compared to controls born similarly preterm but without PV-PPROM; on the other hand, lung diffusing capacity and lung volumes were similar in both EP-born groups. Echocardiographic findings indicated mild persistent PH in the PV-PPROM group. The PV-PPROM group did surprisingly well, especially in terms of lung diffusing capacity, considering their turbulent prenatal and neonatal history and the unfavourable environment in which their lung development took place.

7.1 Methodological considerations

Potential bias related to the studies presented in this thesis will be discussed in the following sections.

7.1.1 Subjects and study design

7.1.1.1 Selection of participants

Optimally, in outcomes research, apart from the exposure being investigated (EP birth and PV-PPROM in the studies presented in this thesis), the study group should not differ from the general population to avoid selection bias. Therefore, studies of outcomes following EP birth and PV-PPROM should be preferably population-based. Norway has a relatively egalitarian societal structure, compared to many other countries, with free access to health care for all children. Despite no active matching criteria regarding social class, no significant differences in educational level were found between the families of EP-born and those of term-born participants.[279] Thus, the risk of bias caused by differences in socio-economic status was likely to be low.

All mothers of EP-born and PV-PPROM participants were residents in a defined geographical area, and to the best of our knowledge, no eligible neonates were born outside this area during the inclusion period with subsequent transfer into the area, which would have affected their study inclusion status Hence, the studies presented in this thesis were truly population-based, and therefore less likely to be influenced by confounding factors, compared to centre-based studies where, for example, high-risk pregnancies tend to be over-represented. Moreover, study loss to follow-up was only minimal, and although the total number of study participants was relatively low, the risk of selection bias was considered to be small.

Studies of this kind are always at risk of survival bias, as by nature, only survivors can be included. Thus, changing survival rates over time, as well as different survival rates between our population and other comparable populations, could potentially lead to bias of the study results. Survival rates following NICU admission in the EP studies were 61% in 1982–5 and 76% in 1991–2, and corresponding number for subjects admitted to NICU at HUS after PV-PPROM in 2000–4 was 76%. These figures are consistent with other reports in comparable populations.[15, 76, 130, 184] However, there were no data regarding stillbirths and deaths in the delivery suite, and therefore the actual total survival rates are unknown. For the PV-PPROM study, unfortunately, data were not available on the number of pregnancies that resulted in intrauterine fetal death, stillbirth, or elective termination at HUS during the inclusion

period due to lack of specific diagnostic coding for PV-PPROM. If previously reported incidence rates[29, 30] of PV-PPROM are applied to the Norwegian perinatal care setting, it can be assumed that the number of fetal deaths must have been high. Thus, differing survival rates can constitute an important source of selection bias in the studies presented in this thesis, potentially favouring positive outcomes in the study groups of participants. However prenatal care, neonatal treatment standards, and survival rates for children born EP in Norway are in line with those in comparable countries,[95] and the neonatal mortality rate of 24% for the admitted PV-PPROM group is similar to rates reported in other studies.[130, 309] This suggests that the study population here is likely to be representative of 'the average perinatal survivor' of PV-PPROM.

To gain better knowledge of the causes of preterm birth and PPROM, ideally, more detailed antenatal and perinatal data on the study subjects should have been obtained. Laboratory results of analysis of maternal cervical samples for microbiology, inflammatory markers, blood samples from the umbilical cord, and placental samples, for example, could be a valuable source of information. However, since data from the pregnancy and neonatal periods were collected retrospectively, it was not possible to gather more data than those already recorded in the 1980s and 1990s for the two EP-born cohorts, and in early 2000s for the PPROM subjects. Prospective studies would be more appropriate to ensure antenatal and perinatal data are recorded and included for analysis. As an example, 'Project Extreme Prematurity' ('BabyPEP') at HUS was designed as a prospective, population-based cohort study.[310]

In many follow-up studies of preterm-born subjects, inclusions were based on BW alone. Although BW and GA are co-linear measures, population samples based on BW alone will necessarily capture a variable and unpredictable proportion of individuals who are SGA. In the EP studies presented in this thesis, the inclusion algorithm focused on preterm birth and GA. However, it is well known that setting the delivery date based solely on the mother's LMP is not fully reliable and, in some cases, can be off in estimating the actual due date by several weeks. In the 1980s, ultrasonography was not routinely performed to estimate delivery dates. To ensure all subjects considered to be born EP within the study time periods were included,

individuals with BW of 1000 g or lower were also included, regardless of the GA. Thus, five EP-born subjects were included based on BW ≤1000 g in the 1982–5 birth cohort, and two in the 1991–2 birth cohort. Although this strategy was a potential cause of selection bias, it was considered preferable to the alternative strategy of inclusion based exclusively on the GA estimated by the LMP.

7.1.1.2 Selection of control groups

A cohort is a group of individuals sharing a certain characteristic, which, in the EP studies presented here, was EP birth. Inclusion of unbiased and representative control subjects is essential for outcome comparisons between those exposed and those not exposed to EP birth and PV-PPROM, respectively. Both EP studies included matched control subjects, which means that each case was individually paired with a control subject. Controls included in the EP studies were term-born, and the matching criteria included age and sex, and BW between the 10th and 90th percentile. For the PV-PPROM study, matching was based on identifying the next-born child of the same sex and GA as the index subject, but without PV-PPROM ≤24 weeks' GA. The rationale was that PV-PPROM should be the only difference between cases and controls. However, PV-PPROM can be caused by a multitude of factors such as infection and inflammation, and it is also associated with low socio-economic status and behavioural factors, such as smoking, that potentially could affect outcomes.[34-36] Nevertheless, similar associations have also been found with preterm birth,[18, 20, 21, 23] and therefore, it can be speculated that both controls and cases were exposed to many similar antenatal factors. As matching was used in the study design, paired statistical analyses were performed.

Selecting representative control subjects is important to reduce bias and maintain high research quality. Some studies include as control subjects medical students, hospital staff, friends, children of colleagues, or school class volunteers. This type of recruitment is associated with an increased risk of a biased control group, which may influence the results and conclusions of a study. For example, a control group could be biased towards a better health status by typically recruiting medical students or healthy hospital staff; conversely, a control group could be biased towards

a poorer health status by recruiting volunteers who may actively choose to participate because of symptoms or other concerns that would give them a high personal interest in being part of a study investigation. In the studies described in this thesis, a strict process for recruiting control subjects was used, based on the 'next-born subject' principle. For the EP birth cohorts, approximately 1.3 controls were invited for each EP-born subject and only one term-born control was excluded for medical reasons (a patient with severe chronic lung disease). Given the strict recruitment process and the low exclusion rate, there is strong reason to believe that the control groups were representative of the general population of term-born survivors.

7.1.1.3 Loss to follow-up

The EP studies were longitudinal, controlled cohort studies, and the PV-PPROM study was a retrospective, population-based follow-up study. A common problem with longitudinal studies is loss to follow-up, and low dropout rates are critical to maintaining high research quality and avoiding biased participation. The proportion of subjects who participated at least once in the studies described in this thesis were 48/51 EP-born survivors from the 1982–5 birth cohort, 35/35 EP-born survivors from the 1991–2 birth cohort, and 11/13 of EP-born subjects who survived hospital treatment after PV-PPROM. Thus, compared to other similar studies,[266, 311] the studies here had high rates of participation, which contributed to ensuring an unbiased study sample.

7.1.1.4 Statistical power calculations

The statistical power reflects the probability of a test/study to correctly reject a false null hypothesis. Power calculations may aid in study design by indicating the appropriate sample size, given that the smallest true difference between groups that would be clinically valuable is provided. A high statistical power reduces the risk of making a type II error (i.e. incorrectly not rejecting the null hypothesis when there is a significant effect), whereas a low statistical power can lead to invalid result interpretations, and thereby incorrect study conclusions. Therefore, the power of a study is commonly required to be between 80% and 90%.

As mentioned earlier, the EP studies were originally designed to detect a difference in FEV_1, and while the study participants were already enrolled, it was therefore not possible to change the size of the study groups. DL_{CO} measurements generally show higher variability, compared to spirometry measurements, at all ages,[247] which may therefore generate higher SDs for DL_{CO}, compared to, for example, FEV_1. Moreover, some participants were not able to perform lung diffusing capacity tests, resulting in even smaller sample sizes for these measurements. These factors limit the statistical power for detecting differences in DL_{CO} measurements between groups. In the BPD subgroup analysis, the groups were even smaller, further lowering the statistical power. Ideally, more participants should have been included to reduce the risk of type II errors. However, a 'near-significant' *p*-value does not automatically become smaller as the data set becomes larger.

External validity is the degree to which the results from a study can be generalized to another similar population. Ideally, the studies included in this thesis should have had more participants. Nevertheless, given the robust study design and the characteristics of the study groups, it is strongly believed that the study results here are valid. Thus, these study data can be used to make inferences regarding outcomes for other preterm-born individuals and those born after PV-PPROM, respectively. Results from the subgroup analysis based on BPD status in the EP studies should be interpreted with caution, however.

7.1.2 Data collection

A researcher's judgement and interpretation of data may be affected by knowing the exposure status, as well as previous measurements, of study participants. Ideally, all physicians and technicians involved in a research study aiming to compare outcomes between exposed and unexposed individuals should be blinded to participants' exposure status. In the studies presented in this thesis, the physicians and technician collecting the data were also researchers involved in data interpretation. Moreover, the physicians involved in data collection were also involved in searching for potential subjects from medical records and registries, contacting these subjects for invitation to participate, and conducting examinations, as well as in data plotting and analysis and

discussions of the study findings. Although only a few physicians were involved in these studies here, none were blinded. This might therefore have contributed to bias.

Another form of bias relates to recall of study participants and their families. Survivors of EP birth and their parents are more likely to recall events that occurred during pregnancy and at birth, as well as during the neonatal period and childhood. They may also be more aware of health-related issues, compared to healthy term-borns and their families. Moreover, there is a risk that those families with a child born EP or after PV-PPROM might have spent more time thinking about the EP birth-related events they have been through and subjective information may, in some respect, be affected by perception rather than by true knowledge. Recall bias is highly relevant and difficult to avoid in the studies included in this thesis. Some aspects of the recall bias may be controlled by using objective data sources when possible. In the studies here, medical records and registries were used to collect data on, for example, neonatal characteristics such as GA and BW, as well as treatment given in NICU, which might have helped to avoid, or reduce, recall bias related to these parameters.

7.1.3 Pulmonary function testing

All pulmonary function tests were conducted in the same pulmonary laboratory at HUS. Standardized equipment were used, and standardized conditions applied, for all measurements and the manufacturer's instructions on equipment maintenance were followed. These factors ensured minimal procedural variability.

7.1.3.1 DL_{CO} measurements

Measurement of SB-DL_{CO} is the most widely used method worldwide to measure pulmonary gas exchange. Measurements were performed according to ERS/ATS standards[230, 286] and the findings here should therefore be comparable to those reported by others. Moreover, the same equipment was used for all measurements at all time points, which reduces the risk of increased variability in measurements caused by using different technical equipment. There is no standardization recommendation available for the IB method. However, the manufacturer's manual of procedures was followed, with the same recommendations for reproducibility of the SB method

applied to test reproducibility of the IB technique.[230] This helps to mitigate the risk of procedural variability and ensured that IB-DL_{CO} measurements were carried out in the same way throughout the studies.

The 2005 ERS/ATS standardization[230] used to define acceptable DL_{CO} tests in the studies here recommends that the inspiratory volume (V_I) should be ≥85% of the largest VC in the same test session. However, their previous standardization from 1993[286] recommended that V_I should be within 10% of the known VC. Because a low V_I could lead to underestimation of V_A and DL_{CO}, the limit for V_I was set to ≥90% of the largest VC in the studies here. Nevertheless, a few measurements with smooth curves within time limits and with V_I below, but close to, 90% of VC were accepted. In the updated 2017 ERS/ATS standardization, the limit for acceptability regarding V_I has been adjusted to V_I ≥90% of the VC or V_I ≥85% of the largest VC in the same test session and V_A within 200 mL or 5% (whichever is greater) of the largest V_A from other acceptable manoeuvres.[247] Storebø *et al*. previously demonstrated that including DL_{CO} measurements with a V_I-to-VC ratio of 0.7–0.85 did not significantly affect results in their study.[312] Therefore, in the studies described in this thesis, the few included cases with a V_I-to-VC ratio slightly below 90% were not considered to have a significant effect on the study results.

For the comparison study between the SB and IB techniques, a true and simple randomization process was used to determine which of the two methods would be performed first. Therefore, there was a 50% chance of either method being selected to be used first. The possibility of a 'learning effect' that could affect the study results was thereby reduced.

DL_{CO} measurements represent a proxy to alveolar function, and not alveolar structure. To fully assess alveolar conditions following EP birth and PV-PPROM, detailed radiological imaging and lung biopsy analysis to describe the alveolar structure could have been performed. However, lung biopsy procedures are associated with a risk of complications such as pneumothorax and vascular events[313, 314]—the study participants here were otherwise healthy individuals. Moreover, one can argue that function, and not structure, most likely constitutes the greatest practical significance for the individual.

7.1.3.1.1 Participation in measurements of DL_{CO}

Participation rates in the studies of DL_{CO} measurements were quite high. However, not all participants were able, or willing, to perform lung diffusing capacity tests, and the proportion who completed DL_{CO} measurements was above 85% in all groups in the EP studies at both follow-ups. In the PPROM study, 8 (73%) of the PV-PPROM subjects and 9 (82%) of the matched control subjects performed SB-DL_{CO} measurements.

A few subjects in the EP studies who either struggled with the technique or lacked motivation did not participate in DL_{CO} measurements at the higher O_2 tension; there are therefore no data for D_M and V_C for these individuals (Figure 2). As there were more EP-born subjects than controls who struggled with DL_{CO} measurements, there is a risk that the attrition might have caused bias in the study conclusions. Moreover, there was a higher percentage of males who failed to participate in DL_{CO} measurements at both O_2 tensions (especially in the 1991–2 birth cohort at the first follow-up where 23% of EP-born males, compared to 4.5% of females, did not perform D_M/V_C measurements), which might have affected the results. However, this was adjusted for by reporting results as *z*-scores (Paper III) and percentages predicted (PV-PPROM study, Paper II) where gender and height were included in the equations.

7.1.3.1.2 Adjustments of DL_{CO}

Besides its variability with age, gender, and height, DL_{CO} is also affected by Hb concentration, COHb level, altitude, exercise, and body position. In the studies here, altitude was considered by calibrating the equipment, and exercise capacity tests were always performed after DL_{CO} measurements. The test subject was seated throughout the whole test procedure to minimize influence by body position. CO binding to Hb is an important factor in DL_{CO} measurements. Anaemia causes a decrease in DL_{CO}. The ERS/ATS suggest that the 'normal' Hb concentration is assumed to be 14.6 g/dL in adult males and adolescents, and 13.4 g/dL in adult females and children under 15 years.[222]

In Paper I,[315] DL_{CO} and K_{CO} were corrected for Hb concentration, measured in venous blood samples from all but five and four participants from the 1982–5 and

1991–2 birth cohorts, respectively. For these nine subjects, the average Hb concentration, calculated for either sex in each subgroup in both birth cohorts, was used in correction calculations. The correction equations presented by the ERS/ATS,[222] based on the work by Cotes *et al*.,[316] were used. Hb concentration was measured also at the 2008–9 follow-up. However, because the majority of study participants had a normal, or near-normal, Hb concentration at both examination sessions (see details in Section 6.2), and values were not too far off the suggested standard Hb values,[222] it was decided to report data uncorrected for Hb concentration in Paper III, in accordance with the GLI reference values for DL_{CO} for Caucasians.[247] Stanojevic *et al*. conducted a study based on a large set of DL_{CO} data from a healthy population, which indicated that there was no difference in *z*-scores calculated using Hb-corrected DL_{CO} reference values versus those calculated using Hb-uncorrected DL_{CO} reference values (mean difference <0.0001).[247] The decision to report uncorrected DL_{CO} data here was based on the factors discussed above; this also helps to avoid introducing further variability to the analyses. Unadjusted DL_{CO} and K_{CO} were also reported in the PPROM study.

Smoking and exposure to high levels of air pollution lead to increased levels of COHb. COHb causes an acute, reversible decrease in DL_{CO} by both competing for Hb binding sites, leaving fewer available binding sites for CO in the test gas, and reducing the CO driving pressure gradient from alveolar gas to capillary blood. It is therefore recommended that test subjects should refrain from smoking on the day of lung diffusing capacity testing.[222] Study participants here received this instruction by letter ahead of the scheduled test appointment. Self-reported smoking was verified in the 1982–5 birth cohort at age 18, but not 25, by measuring urinary cotinine levels; three positive tests (5%) were obtained among 57 self-declared non-smokers.[207] The same confirmation of self-reported smoking was not carried out in the 1991–2 birth cohort. COHb levels in blood were not measured, so the possibility that some subjects had smoked on the test day without reporting it cannot be fully excluded, which might have impacted the results.

Exposure to high levels of air pollution is rarely an issue in Bergen where the studies described in this thesis were performed, so data on exposure to air pollution

were not obtained for the studies here. The ERS/ATS also recommend adjusting for COHb only when COHb levels are known or suspected to be elevated. Although it cannot be fully ruled out that air pollution might have affected measurements in some of the participants, it was considered that its effects, if any, most likely were too minimal to influence the study results.

According to previous studies, DL_{CO} varies during the menstrual cycle in females, with the highest values just before the menses and the lowest during the menses.[234] In the study relating to the GLI reference equations for DL_{CO}, Stanojevic *et al*. found that the CV was higher in adult females than in adult males, but only minimal differences in children aged <10 years and older females aged >55 (who are presumably post-menopausal).[247] The authors therefore argued that the higher CV seen in adult females may be related to the menstrual cycle. Data on the menstrual cycle in adult females included in the studies presented in this thesis were not recorded. The child participants (the 1991–2 birth cohort at the first follow-up, and the PV-PPROM study participants) were all aged around 10 years at the time of examination and most were presumably examined before reaching menarche, which occurs at a mean age of around 13 years in Norway.[317] It was therefore concluded that variation of DL_{CO} related to the menstrual cycle was unlikely to affect results in these subjects, although some degree of impact on DL_{CO} data from the older subjects could not be excluded.

7.1.3.1.3 Reference equations

In general, reference equations are typically based on data from healthy subjects with the same anthropometrics (including sex, age, height, ethnicity).[318] Access to reference values for different pulmonary function tests facilitates comparison of results obtained from patients and study participants tested in our laboratory with predicted values, thereby identifying any changes and deterioration that are outside the normal variability.

Absolute values obtained from a pulmonary function test may be expressed either as percentages of the values predicted or as *z*-scores. The *z*-score is the number of SDs the obtained data deviate from the expected mean value given by the reference

equation. The percentage predicted is associated with age- and height-related bias and should therefore be used with caution. On the other hand, *z*-scores take into account age, height, sex, and ethnicity, as well as the age-dependent reference range, and are therefore increasingly used.[319] Thus, an advantage of using *z*-scores is that any given *z*-score indicates comparable lung function between individuals, irrespective of their sex, height, age, or ethnicity. The *z*-score also enables bias-free interpretation of serial measurements within a person during growth and ageing, which is a clear advantage, especially for paediatric populations during growth.

Even though the lung diffusing capacity test is one of the most common pulmonary function tests in use, next after spirometry, traditionally there has not been any international consensus regarding use of reference equations, primarily most likely because of higher inter-laboratory variability of DL_{CO}, compared to spirometry parameters.[318] Additionally, laboratories have used different equipment and techniques, thus complicating the development of international reference equations. Reference values for DL_{CO} for the paediatric population have been even more scarce. There are a few published studies presenting predicted values for DL_{CO} in children.[320-323] However, their results were based on using several different techniques, some of which have become obsolete. Further, some of the reference equations were based on relatively old data. Changes in equipment, software, and measurement techniques in combination with a shift in population characteristics imply that some of those reference equations therefore may no longer be valid. Another limitation with using separate equations for children and adults is that it may lead to discontinuities in the interpretation of results. Therefore, in 2017, the GLI published reference equations for DL_{CO} measurements for Caucasians aged 4.5–91 years, with a median age of 45 years.[247] Data from 9710 healthy subjects (of whom around 50% were female) were included. Paediatric data included in the analysis were based on two studies conducted in the past decade.[242, 324] DL_{CO} values included in the reference material were corrected for altitude and fraction of inspired O_2 (FiO_2), and uncorrected for Hb concentration.[243]

Papers I and II were published before the GLI reference equations became available. Lung diffusing capacity data in these two papers were presented as raw data

or percentage predicted. Data in the longitudinal study described in Paper III were reported as *z*-scores based on the GLI equations. This proved advantageous, as it allowed use of the same equations for all included subjects at both time points. The GLI reference equations are, to this day, only valid for Caucasians. However, this did not constitute a major limitation for the studies presented here, as the vast majority of the participants were Caucasians. The GLI equations were updated in October 2020 as it was recognized that females with low DL_{CO} scores had exceptionally low *z*-scores when calculated using the GLI DL_{CO} calculator.[248] Therefore, data reported in the studies presented in this thesis were adjusted accordingly.

7.1.4 Data preparation

Antenatal and neonatal data were retrieved from hard copies of obstetric and paediatric records for the two EP-born cohorts. Medical records were available for all but one subject in the 1982–5 birth cohort. Information from both the discharge summary and maternal recall was therefore used as information sources for this individual. For the PV-PPROM study, data were gathered from medical records and parental questionnaires.

Raw data from pulmonary function tests, anthropometric data, clinical examination findings, and results from analysis of blood and urine samples were manually entered into a data file. To avoid mistakes, all data were entered twice.

It was therefore concluded that both data collection and preparation were satisfactory in terms of quality.

7.1.5 BPD definition

The current definition of BPD is based on the need of supplemental O_2 for >28 postnatal days and the grade (mild, moderate or severe) is determined at 36 weeks' PMA.[102] While such a 'yes/no' definition is easy to use in research settings such as in the studies here, it is less useful in clinical settings as it does not reflect contemporary neonatal care and does not adequately predict childhood morbidity.[105] The current definition does not differentiate between the forms of ventilatory support given, and new modes of ventilatory support, such as n-CPAP and NIPPV, are not accounted for.

For example, children treated with very low-flow 100% O_2 or high-flow room air (21% O_2) via nasal cannula may be difficult to classify according to the current standard definition, with the risk of leaving some infants unclassified. Moreover, the current definition fails to correctly classify infants who die from respiratory failure before 36 weeks PMA.[104] Consequently, these infants may not be included in some studies where clearly their inclusion would have been beneficial to help in answering important clinical and research questions on BPD.

Most NICUs do not have a standardized method to define 'need of supplemental O_2', and the criteria for O_2 administration may vary among different physicians, as well as among centres. There are also different views regarding O_2 saturation targets, and use of different saturation targets may affect the concentration and duration of O_2 supplementation. Consequently, these factors may lead to differences in BPD diagnosis. The 'room air challenge' was first introduced in 2003 in an attempt to provide a physiological basis for diagnosing BPD, rather than relying on individual physicians' clinical assessment.[325] However, this method has not been adopted consistently across NICUs, and BPD diagnosis is therefore still often based on an individual physician's clinical evaluation.

Changing BPD definitions poses a problem when comparing long-term effects and may confound interpretation of results. The same definition was used for both EP-born cohorts included in the studies presented here.[102]

New refinements to the existing BPD definitions—based on persistent parenchymal lung disease, radiographic confirmation of parenchymal lung disease, and O_2 requirement at different levels (via invasive IPPV, n-CPAP, NIPPV, or nasal cannula) at 36 weeks' PMA—were proposed by the NICHD in 2018.[104] The future will tell if another new definition of BPD would be available that is hopefully better suited for both clinical work and research purposes.

7.2 Discussion of main study results

7.2.1 Study aim 1(i): Reproducibility of SB-DL_{CO} measurements in children (Paper I)

Generally, the aim of performing pulmonary function tests is to distinguish normal (health) from abnormal (disease), or what we, as clinicians, regard as a significant deterioration from what is normal. In daily clinical practice, this is important as it has bearing on our decision-making in terms of whether to treat and follow up a patient, and if so, how. All types of pulmonary function tests are subject to different types of variations, including: (1) technical variation relating to equipment, procedure, and instructions given by the technician; (2) biological variation; and (3) variation due to impairment or disease.[326] Determining the reproducibility of DL_{CO} is fundamental to being able to distinguish true differences in DL_{CO} as a result of procedural or normal biological variation. Knowing the reproducibility of a measurement in both normal, healthy subjects and patients with disease or known impairments will further enhance the interpretation of the results.[327]

DL_{CO} measurements are frequently reported in studies of EP-born children and adults and are also used in different clinical settings in paediatric departments worldwide. Studies have reported on inter-session variability for DL_{CO} measurements in adults.[277, 328, 329] However, to our knowledge, data on reproducibility of DL_{CO} measurements in children are lacking. Moreover, it is unknown whether reproducibility in individuals born EP differ from that in healthy controls, as EP-born subjects may have a higher incidence of cognitive, sensory, and/or physical impairments that can make DL_{CO} testing more challenging for these individuals. It is therefore useful to study the reproducibility of DL_{CO} measurements for children, as well as for EP-born subjects. Results showed that the inter-session CV for SB-DL_{CO} measurements for the 10-year-old subjects was similar to that for healthy adults (represented by the control group in the 1982–5 birth cohort). Therefore, it can be reasonably assumed that the SB-DL_{CO} procedure can be used in follow-ups of individuals during mid-childhood. This is important knowledge for clinicians and technicians who are involved in pulmonary function testing of children in different clinical settings, and not only of individuals who survive EP birth. Moreover, results

also showed that EP-born subjects were able to perform SB measurements with acceptable reproducibility, compared to term-born controls, and below the cut-off of 10% discrepancies for significant differences set by the ATS/ERS.[222]

Paediatric DL_{CO} data included in the development of the GLI reference equations showed that between-subject variability was considerably higher in children than in adults.[247] Generally, when performing DL_{CO} testing in children, instructions must be kept as simple as possible, with the instructor often needing to demonstrate the manoeuvre, and encouragement is important throughout the test. Moreover, the child needs to be able to coordinate breathing according to the instructions given and maintain focus throughout the test, while at the same time avoiding to do a number of things as instructed before the test (such as refraining from performing the Müller or Valsalva manoeuvre, avoiding gas leaking which can occur if the test subject does not keep their mouth tightly closed around the mouthpiece, and staying in an upright position throughout the test). All these factors may prove more demanding for a child, and especially children with any form of cognitive impairment, which may possibly explain the higher variability seen for DL_{CO} in children than in adults. These factors may also increase intra-session and inter-session variability in children. Because between-individual variability naturally will be higher, compared to within-individual variability, the CVs presented in the GLI publication by Stanojevic *et al*. would be expected to be higher (as seen in Figure 2 in the article,[247] with CVs of between 15% and 20% for 10-year olds) than those reported in the studies described in this thesis. Another small study demonstrated within-individual, intra-session variability (repeatability) among children, presented as a CV of around 2.5%.[251] The CVs for repeatability of DL_{CO} among adults have been shown to be around 3.1% in healthy subjects and around 4% in subjects with abnormal spirometry patterns,[330] while the 90th percentile for mean long-term inter-session variability in middle-aged adults have been reported to range between 10.9% and 15.8% (2.6–4.1 DL_{CO} units).[277] Data from studies included in this thesis showed CVs ranging from 7.5% to 9.6%, with mean percentage differences varying from 1% to 5.4%, which are thus higher than in the repeatability studies,[249, 251] but lower than in the long-term inter-session study[277]— this is expected and the study data here therefore are considered valid and adequate.

According to previous reports, subjects with pulmonary obstruction were shown to have higher mean CV, compared to those without any respiratory impairment.[330] Our research group has previously shown that EP-born subjects in our cohorts have a higher degree of airway obstruction,[199] compared to control subjects, which may have contributed to the higher CV seen in EP-born subjects in both birth cohorts, compared to term-born controls. Another possible reason for the higher variability observed in the EP-born groups could be a higher prevalence of mild cognitive and sensory impairments which may influence measurements.

Values of SB-DL_{CO} and SB-K_{CO} obtained on the first assessment day exceeded those obtained on the second day for all subgroups in both birth cohorts. This is a surprising finding, with no obvious explanation. The same equipment was used on both test days, together with the same standardization protocol, and the same experienced technician provided the same guidance during all the tests. However, some form of unknown technical issue cannot be ruled out. One may also speculate that the study participants possibly experienced a higher degree of anxiety on day 1, which would have caused the heart rate to increase, which, in turn, would have caused an increase in pulmonary blood flow, and consequently DL_{CO}. Another possible explanation could be that the participants were more unfamiliar with the test procedure on day 1, such that they could have performed some degree of the Müller manoeuvre during the procedure, which would have increased intrathoracic blood flow, resulting in increased DL_{CO}. However, these are only speculations, and due to the small numbers of participants, the higher values of SB-DL_{CO} and SB-K_{CO} on day 1 could very well simply be random. Larger studies that include a higher number of study participants would have made it possible to better explore this issue.

Even though reproducibility varied somewhat between the different subgroups and was higher among EP-born subjects, the overall differences between measurements were quite small and below the 10% cut-off set by the ERS/ATS[222] for detecting clinically relevant differences. Therefore, it can be concluded that the ability to distinguish health from disease, in other words the reliability of the method, was adequate in both children and EP-born subjects.

7.2.2 Study aim 1(ii): Comparison between the SB and IB methods of measurement (Paper I)

Values of IB-DL_{CO} were lower than DL_{CO} values obtained with the SB technique. In the SB method, gas diffusion takes place at TLC during the breath-holding time of 10 seconds, whereas with the IB technique, gas diffusion occurs as lung volumes are being gradually decreased throughout the exhalation period. The surface area available for gas exchange occurring during exhalation is therefore smaller. This is supported by lower V_A measured with the IB technique than with the SB technique. During the SB test, on the other hand, there is possibly a more complete distribution of CH_4 due to the long breath-holding period, which may explain the higher measured V_A obtained with the SB method. Lower values of IB-DL_{CO} and IB-V_A, compared to SB-DL_{CO} and SB-V_A, were therefore expected results. With lower values of both IB-DL_{CO} and IB-V_A, the K_{CO} obtained with both the SB and IB techniques was similar. This illustrates the importance of reporting not only DL_{CO} or K_{CO}, but also all variables, including DL_{CO}, V_A, and K_{CO}, so results can be adequately interpreted from lung diffusing capacity measurements.

Existing literature on comparison between the two methods of DL_{CO} measurements is sparse and somewhat contradictory. Wilson *et al*. compared IB-DL_{CO} to SB-DL_{CO} in an adult population, with findings in agreement with the results from the studies in this thesis,[229] whereas other studies reported conflicting results.[331, 332] Kiss *et al*. described higher mean V_A values obtained by the IB method, compared to the SB technique, in all 100 adult study participants, whereas DL_{CO} differed between patients with and those without airway obstruction. Patients without and those with airway obstruction, respectively, had higher and lower IB-DL_{CO} values, compared to SB-DL_{CO}.[331] Both EP-born and term-born subjects in the studies discussed in this thesis had lower DL_{CO} values with the IB method than with the SB method, even though a higher proportion of EP-born subjects had airway obstruction, compared to term-born controls. These results do not correlate with the study findings of Kiss *et al*.[331] By contrast, another study comparing the two techniques in patients undergoing heart transplantation found results in line with those reported by Kiss *et al*., with higher V_A values obtained with the IB technique than with the SB technique.[332] These

two studies demonstrated a better correlation between IB-V_A and measured TLC, compared to the SB values.[331, 332] Because results from the different studies are not one-sided, it is difficult to properly assess the differences and similarities between the two techniques.

Overall, results from the studies in this thesis and the other mentioned studies[229, 331, 332] indicate that the IB technique appears to produce results that are fairly comparable to those obtained with the SB method. However, although the IB technique is theoretically useful for patients who have difficulty with the SB-DL_{CO} procedural manoeuvre, such as younger children and dyspnoeic patients who have difficulty breath-holding for 10 seconds, its clinical relevance is hampered by a lack of standardization. Another issue with the IB technique is that reference equations are lacking, making result interpretation difficult. Most pulmonary laboratories worldwide use the SB method to assess DL_{CO}, which makes it easier to compare results across different other centres. To conclude, therefore, the SB technique should be preferably used in all individuals who are able to perform the procedure, while reserving the IB technique only for those who would find the SB technique too demanding and challenging to perform and where DL_{CO} measurements are clinically relevant.

7.2.3 Study aim 2: Comparison of lung diffusing capacity between subjects born EP and term-born controls (Papers I and III)

In the studies described in Papers I and III, comparing EP-born subjects with term-born controls, lung diffusing capacity was significantly reduced following EP birth. DL_{CO} was reduced by approximately 12–18%, and K_{CO} by approximately 7–10%, in EP-born subjects.

Results from Paper III[333] showed that the mean *z*-score differences for DL_{CO} between EP-born subjects and term-born controls ranged between 0.6 and 0.9 (data from both time points). Based on the observed variability of DL_{CO}, the GLI identified 0.5 *z*-scores as a threshold for a physiologically relevant difference regarding DL_{CO}, corresponding to approximately 0.3–0.8 $mmol \cdot min^{-1} \cdot kPa^{-1}$ or 10% relative change in DL_{CO}.[247] The differences obtained in the study here are therefore above the 0.5 *z*-score threshold. Thus, from these results, it can be inferred that there are most likely

true differences with generally reduced DL_{CO} in EP-born subjects, compared to term-born controls. Similar findings in both birth cohorts that persist from the first to the second examination strongly supports this, suggesting these differences are less likely to be due to arbitrary variability.

Further, these findings are in line with those reported from comparable studies of EP-born subjects.[193, 198, 261, 264-268, 271, 311] It should be noted that lung diffusing capacity parameters are reported in various ways in these referred studies, including *z*-scores, raw data, and only percentages predicted, and not all variables (DL_{CO}, K_{CO}, and V_A) are consistently reported. It is therefore difficult to calculate the exact magnitude of the differences between EP subjects and controls. However, it would appear that mean differences generally are around 10%.

Depending on the GA at birth, neonates born EP spend parts of the second trimester and the whole third trimester outside the uterus. Important developmental processes in the lungs, including the first phase of alveolarization, must occur while the neonate is being treated in NICU. In parallel with the neonate receiving lifesaving respiratory support and advanced intensive care, new alveoli are being formed, with gradual expansion of the pulmonary capillary bed. At the same time, these immature neonates are often exposed to dramatic events and consequences associated with EP birth such as infections, NEC, and IVH- all of which completely change the premises under which this developmental programme must take place. Autopsy studies of children who died from BPD showed impaired acinar development,[153, 157, 165, 255] but except for a few case reports,[157, 158, 160] little is known about the detailed lung structural features in survivors. Moreover, abnormal lung structural findings from such studies may not necessarily be representative of the whole EP-born population, as data often come from individuals with the most severe lung impairments.

Even though studies, including those presented in this thesis, examining long-term consequences of EP birth have reported persistently reduced DL_{CO}, these reductions are relatively modest, considering the quite dramatic start in life that these EP-born individuals experienced. Given the relatively significant structural abnormalities reported in histopathology studies, one would expect that lung diffusing capacity would be even more affected than reported in the studies presented here and

by others. DL_{CO} is measured at rest, and with the relatively large ventilatory reserves in healthy individuals, one can argue that the measured DL_{CO} values do not necessarily reflect impairments that may be influenced by physical activity, when the blood transit time through the lung capillaries is shortened. However, we previously demonstrated that these same EP-born subjects had near-normal peak exercise capacity, compared to term-born controls.[300] These findings have been replicated also by others.[265, 334-336] Thus, results from both DL_{CO} measurements and exercise capacity testing complement each other and suggest that acinar impairments following EP birth might not affect physiological function as severely as one would anticipate.

Although EP-born subjects as a group had *z*-DL_{CO} above the cut-off set by the GLI for physiologically relevant differences with mean impairments of around 10%, compared to control subjects, these findings are far off the limit set by the American Medical Association (AMA) regarding assessment of pulmonary dysfunction. In the AMA guidelines, DL_{CO} is included as one of the parameters in the assessment, whereby Class 0 (no symptoms and/or intermittent dyspnoea, and no current signs of disease) is defined as DL_{CO} ≥75% of predicted, Class 1 dysfunction as DL_{CO} 65–74% of predicted, and Class 4 (the highest severity class) as DL_{CO} below 45% predicted.[337]

Reduced DL_{CO} seen in EP-born children are due to reductions in the membrane component and/or pulmonary capillary factor, which will be discussed further in Section 7.2.4.

7.2.3.1 Lung diffusing capacity in relation to BPD

BPD did not influence lung diffusing capacity variables (*z*-DL_{CO}, *z*-K_{CO}, and *z*-V_A) in the EP studies, and EP-born subjects with a history of BPD surprisingly had higher *z*-DLCO, compared to EP-born subjects without BPD. Collard *et al*. previously demonstrated that airway obstruction can lead to increased DL_{CO}.[242] EP-born individuals have been shown to have persistent airway obstruction, [199, 256] as also in the cohorts in the EP studies here. Thus, airway obstruction might theoretically counteract DL_{CO} deficits caused by acinar impairment. This can possibly explain the finding that EP-born subjects with a history of neonatal BPD and more severe airway obstruction had higher *z*-DL_{CO}, compared to those with no BPD and less severe airway

obstruction. However, given the small study sample size, no clear conclusions can be drawn on this somewhat surprising finding. It is of interest that a seemingly similar lack of influence from BPD on lung diffusing capacity has been observed also by others. [261, 266]

7.2.4 Study aim 3: Longitudinal development of K_{CO} and DL_{CO}, and its subcomponents from mid-childhood to adulthood (Paper III)

7.2.4.1 Longitudinal development of DL_{CO}, V_A, and K_{CO}

Both DL_{CO} and K_{CO} were persistently reduced following EP birth, from mid-childhood and up to young adulthood. Results also showed that development tracked below, but parallel with, that of term-born controls. No signs of early-onset decline of DL_{CO} or K_{CO} were observed in the EP-born group at age 25 years. However, there were also no obvious signs of pubertal catch-up growth. As mentioned in the introduction section 1.2.2, MRI studies showed continued alveolarization to adolescence and catch-up growth following EP birth.[68, 69] These studies provide optimism that repair mechanisms might have an impact as these EP-born individuals grow. However, judged by the findings presented in Paper III, there were no signs of a corresponding functional catch-up in the EP-born subjects included in the study.

To our knowledge, there are no previous studies presenting longitudinal data on DL_{CO} following EP birth. However, cross-sectional studies of EP-born subjects at different ages from mid-childhood to early adulthood have yielded similar results, with varying degrees of reduced DL_{CO}, compared to control groups.[193, 198, 261, 264-268, 271, 311]

Further, in the study here, *z*-scores for DL_{CO} increased for all subgroups from the first to the second follow-up, also during early adulthood from age 18 to 25 years. Based on the information presented in Figures 1 and 4 from the GLI article by Stanojevic *et al*. for reference values for DL_{CO},[247] data included in their analysis indicate that absolute DL_{CO} is at its maximum at around age 20–25 years. The development seen from age 18 to 25 years could be explained by increased thoracic width, especially in males, and by concurrent increase in alveolar size. However, as age, sex, and height are taken into account when calculating z-scores, one would expect that none of these factors would affect the *z*-scores, thereby leading to increased

z-scores during the observed time. Although there is no obvious explanation for this, one can speculate that the reference equations might not be applicable to the study population here or that the increase in *z*-scores was arbitrary.

There are concerns regarding the future pulmonary health of EP-born individuals, as many seem to fail to reach their optimal lung function in early adulthood. Our research group and others have previously demonstrated that EP-born subjects have significant airway obstruction from mid-childhood to young adulthood.[199, 338] Normal ageing implies a gradual decline in lung function from around age 25–30 years. The finding that EP-born subjects do not reach as high lung function levels as term-born controls is a cause for concern regarding whether these EP-born individuals will follow a normal or steeper decline in lung function during adulthood, and potentially develop COPD. As discussed in Paper III, DL_{CO} is used in clinical practice to assess severity and prognosis of COPD, as spirometry alone poorly reflects the disability in these patients. Reduced DL_{CO} is a prognostic marker, independent of forced spirometry, in COPD patients,[276, 339] and is associated with increased morbidity across multiple domains as well as increased mortality.[276, 340] Moreover, lung diffusing capacity has been shown to be a significant predictor of all-cause mortality within a general population, independent of standard spirometry measures, and even in the absence of apparent clinical respiratory disease.[341]

As mentioned earlier, impairments in lung diffusing capacity among EP-born individuals persist through childhood to young adulthood, indicating similar tracking for DL_{CO} that was previously demonstrated for FEV_1. As discussed in Section 2.1.2.4, DL_{CO} normally starts to decline at some point after age 20–25 years, and subsequently follows an accelerated course after around age 40 years. These findings raise concerns about whether there could be a continuing decline in DL_{CO} in EP-born subjects with increasing age that would gradually lead to impairments that are severe enough to have clinical consequences for these individuals. As smoking is an established risk factor for a more rapid decline, EP-born subjects who smoke early in adulthood, or already during adolescence, are likely particularly vulnerable. Study findings here suggest that one should consider including DL_{CO} measurements when following up individuals with a history of EP birth. Moreover, any physician who encounter these EP-born

individuals in their practice, including paediatricians encountering EP-born teenagers and general practitioners and pulmonologists encountering EP-born adults, should remember to ask about their neonatal history as part of medical history taking, and particular attention should be focused directly on their smoking habits, with guidance provided on choice of occupation to avoid unnecessary exposure to airway irritants, which can further negatively impact lung function.

Theoretically, z-scores for term-borns should, on average, be zero. However, this assumes that the subjects in the study here had the same characteristics as the population from which the reference values were obtained (in other words, same inclusion criteria, same ethnicity, etc.). The rather small deviation from zero seen for the controls might be due to some unknown discrepancies between the reference material and the study population, as well as due to the rather small study sample size.

The study design here has some limitations. Data from two examinations were used to draw conclusions regarding the trajectories from mid-childhood to early adulthood. The study participants were only examined twice and were not followed up throughout the whole period from age 10 to 25 years. As the second examination of the oldest subjects (from the 1982–5 birth cohort) was carried out at around the time point when lung diffusing capacity would be expected to be at its peak (at around age 20–25 years), it would be interesting to follow these same individuals further. This would enable studies to determine whether the normal and expected decline with increasing age follows the development seen in healthy term-born controls or, in the worst case scenario, an accelerated decline over time.

7.2.4.2 Longitudinal development of D_M and V_C

While there is relatively solid evidence for persistent reduced lung diffusing capacity in EP-born individuals from childhood up to young adulthood, the mechanisms underlying the impairments are unclear. Relating back to Fick's law,[221] lung diffusion can be hampered following EP birth due to reduced surface area accessible for gas exchange, thickening and/or impairment of the membrane component of the alveolar–capillary membrane, and/or impaired vascular components. The study presented in Paper III therefore aimed to elucidate these underlying mechanisms by measuring the

subcomponents of DL_{CO}, i.e. D_M and V_C. D_M was numerically reduced at both measuring points in both EP-born birth cohorts, although significantly so only at the first examination in the younger birth cohort (1991–2). Data for V_C did not reveal corresponding deficits. The vascular component increased over time in both birth cohorts, also from age 18 to 25 years, indicating similar growth and development in the EP- and term-born groups, presumably in parallel with increasing body size.

Few studies have reported on the subcomponents of DL_{CO} following EP birth, which means there are only sparse data available for comparison with results from the study described in Paper III. Moreover, these previous studies used slightly different methods to those used here, and examined different age groups, making direct comparison of results even more challenging. A French study from 2020 reported non-significant differences in DL_{CO} and similar D_M, but significantly reduced V_C in adolescent preterm-born subjects, compared to healthy term-born controls. [273] More in line with study findings presented in Paper III, Sørensen *et al*. found reduced DL_{CO} in school-aged survivors of EP birth,[267] with reduced D_M and similar V_C by using a combined DL_{NO}/DL_{CO} method. Chang *et al*. found that both D_M and V_C were reduced in preterm infants and toddlers, compared to healthy term-born controls.[258] Results shown in Paper III thus are not quite in agreement with these findings, nor with the study by Drummond *et al*.,[273] especially regarding the V_C component. However, Chang *et al*.'s study included considerably younger patients (mean age of preterm participants 17.4 months), with measurements performed under sedation by using a different method—again making direct comparison of results difficult. Also, in contrast to the study presented in Paper III, Chang *et al*.'s study only included preterm subjects with BPD, and thus possibly with more severe lung disease. Of note, the study by Drummond *et al*. also used the DL_{NO}/DL_{CO} method.[273]

As alveolarization and the pulmonary vascular components continue to develop during childhood, and possibly all the way through adolescence, one can speculate about whether catch-up development of alveolar components in EP-born subjects could have contributed to the modest differences observed in the study presented here, compared to the findings by Chang *et al*. However, the sparse data from the three studies referred to earlier [258, 267, 273] regarding the subcomponents of DL_{CO} following

preterm birth are clearly ambiguous. Nevertheless, all these studies constitute new, interesting views on alveolar function following EP birth, and highlight how physiological assessments, such as measurements of DL_{CO} subcomponents, may be used in a clinical setting to provide more detailed information on gas exchange. Results from these measurements can be further combined with radiological imaging, for example, to improve evaluation of EP-born subjects on both individual and group levels. As commented also by Chang *et al*.,[258] longitudinal studies are required to determine whether preterm infants exhibit catch-up alveolar development or have persistent deficits. Longitudinal studies including measurements of the subcomponents D_M and V_C at earlier ages than in the studies presented in this thesis would therefore be interesting.

A study limitation with measurements of D_M and V_C is that reference equations, as well as information on normal development from childhood, through adolescence, up to adulthood, are lacking (from personal written correspondence with Professor Sanja Stanojevic, May 2020). Results are therefore difficult to interpret when determining whether the differences seen following EP birth, compared to term-borns, deviate from normal variation. Moreover, the clinical relevance of the measurements is unclear but may nevertheless help in adding one more piece to the jigsaw puzzle of understanding the alveolar consequences of EP birth.

7.2.5 Study aim 4: Cardiopulmonary outcomes in children surviving PV-PPROM (Paper II)

Many expecting parents who have experienced PV-PPROM would have received pessimistic antenatal information regarding expected outcomes and prognosis for their child, with numerous pregnancies terminated. Studies presenting long-term outcomes after PV-PPROM are few, and clinicians worldwide therefore lack reliable information that would help in counselling these parents. Based on their clinical experience and given major advances in modern neonatal intensive care, together with increased availability of a wider range of treatments, including inhaled NO, some of the experienced neonatologists at HUS have suggested that the outcome of infants who survive PV-PPROM is not uniformly poor. Considering the events to which these

individuals were exposed already during fetal life, coupled with the often quite aggressive treatment they received during the neonatal period, results from the study presented in Paper II are mainly encouraging and support the notion suggested by these neonatologists.

7.2.5.1 Airway obstruction

A higher proportion of individuals surviving PV-PPROM demonstrated airway obstruction, compared to preterm controls. We[199] and others[264] have shown that EP-born subjects have increased, but relatively mild, airway obstruction. Results reported in Paper II are in line with these previous findings and suggest that individuals exposed to PV-PPROM are more prone to developing obstructive airways disease, compared to those born preterm with intact membranes. This can probably be explained by resultant oligohydramnios, as amniotic fluid is highly important for fetal lung development.[342, 343] Moreover, the PV-PPROM survivors received more intensive respiratory support during the neonatal period, as reflected by increased number of days on ventilation and CPAP, and a considerably longer period of O_2 supplementation, thus contributing adversely to persistent lung disease. This line of reasoning is supported by a recent meta-analysis which found that BPD was negatively associated with decreased expiratory air flow rates and volumes during late adolescence and early adulthood.[256]

7.2.5.2 Pulmonary hypertension, lung diffusing capacity, and exercise capacity

In the study presented in Paper II, echocardiographic assessments demonstrated mild PH in PV-PPROM individuals, in line with previous studies reporting that PPROM, oligohydramnios, and pulmonary hypoplasia are contributing factors to the development of PH in premature infants.[146] Postnatal factors, such as mechanical ventilation and inflammation caused by infection or other exposures, have also been implicated as risk factors for developing PH.[344] The development of PH may be explained by the fact that neonates surviving PV-PPROM are exposed to many of these mentioned risk factors, while at the same time their extreme prematurity itself

causes arrested development of the pulmonary microvasculature, which results in increased PVR.

Preterm infants with a history of PPROM are more likely to be particularly prone to developing PH.[146] Subjects surviving EP birth, and especially those with BPD, are suspected to be at increased risk of COPD in later life. Patients with COPD are at increased risk of developing elevated pulmonary arterial pressure and PH, and COPD patients with PH have increased morbidity and mortality rates, compared to those with COPD alone.[274-276] Even in mild COPD, there are significant abnormalities in pulmonary microvascular blood flow that worsen disease progression.[219] Since both prematurity and COPD are associated with an increased risk of PH, one can speculate about whether even those EP-born subjects who showed no signs of PH during infancy or childhood are at risk of developing PH as adults. Based on the same reasoning, one can also speculate about whether PPROM survivors are at even higher risk, and whether those who were diagnosed with PH since childhood are at increased risk of developing more severe PH during adulthood.

Lung diffusing capacity was not significantly different between the two study groups. However, there were few study participants and not all performed DL_{CO} measurements, thus leaving only few measurements available for analysis. Caution is therefore advised when drawing conclusions based on these study findings.

Adult subjects with PH have been shown to have reduced DL_{CO}.[274] Moreover, reductions of DL_{CO} in PH patients have also been shown to be associated with increased severity of PH, although no correlation has been found between other pulmonary function tests such as flows and volumes and PH severity.[274, 275] DL_{CO} measurements may therefore help to identify patients with PH whose condition deteriorates despite having stable lung volumes, and it has been suggested that PH should be suspected in patients with chronic lung disease where DL_{CO} is disproportionately low in relation to other lung function values.[274] Reduced DL_{CO} has recently been demonstrated to be an independent predictor of death in patients with COPD and PH.[275] The study authors concluded that DL_{CO} was a robust marker of disease severity in the included patients and that the association may reflect an early reduction in capillary density.[275] It has previously been suggested that a large

proportion of the pulmonary vasculature must be obliterated before pulmonary artery pressure rises to levels where a diagnosis of PH can be made; this means that a PH diagnosis is thus often made late and at a point when the vascular disease is advanced.[345] The widely available and non-invasive DL_{CO} measurements may therefore theoretically be a potential alternative to detect early pulmonary vascular disease ahead of other cardiopulmonary circulation measurements.[275] Measurements of DL_{CO} in EP-born subjects, and especially in survivors of PPROM, could thus be of clinical interest. A possible approach could be that those with reduced DL_{CO}, and especially individuals with an abnormal decline in DL_{CO}, preferably should be assessed by echocardiography for signs of PH.

The subjects included in the EP studies were unfortunately not examined by echocardiography at the two examination sessions, and therefore, no data are available on measurements related to PH for these subjects. However, another study is currently under way involving a third examination of these same individuals that includes echocardiographic assessment. Thus, this study will help to explore the occurrence of PH in these adult individuals, and possibly also relate these findings to DL_{CO} measurements in the future.

As discussed previously, DL_{CO} was measured at rest, whereas impairments in gas exchange may become more evident when ventilation is challenged during exercise. Exercise capacity testing may therefore complement DL_{CO} measurements and provide us with more information regarding gas exchange in these individuals. Peak VO_2 expressed in $mL \cdot kg^{-1} \cdot min^{-1}$ was significantly reduced in the PV-PPROM group, compared to matched preterm-born controls. However, peak VO_2 expressed in percentage predicted was similar between the two groups, and within normal range, as defined by the applied reference equation.[298] Moreover, the values were comparable to those in 10-year-old EP-born subjects from the 1991–2 birth cohort.[301] Thus, while there were differences between the groups, impairments were small and the clinical relevance of these findings questionable, as the overall exercise capacity should be adequate for normal childhood physical activity.

The study results are encouraging and indicate that, despite the presence of airway obstruction, the development of mild PH, and small differences in peak VO_2,

compared to EP-born controls, survivors of PV-PPROM seem to have adequate cardiorespiratory capacity in mid-childhood. Future studies that include more participants are needed to further evaluate the long-term consequences of PV-PPROM. Longitudinal studies of these individuals could provide further insight. The heterogeneity in the approach to patient management at different centres, in combination with these rather encouraging results regarding outcome in mid-childhood, highlights the importance of future studies on maternal and neonatal outcomes following PV-PPROM to guide clinicians involved in antenatal counselling. Moreover, individuals surviving PV-PPROM, as well as their families, deserve that health personnel involved in decision-making about pregnancy termination versus proceeding with lifesaving intensive treatment have evidence-based knowledge to inform and support their decision-making, and importantly they deserve to know what prognosis and future can be expected for these vulnerable neonates.

8 Conclusion

Below are the interpretations of the main findings of the studies presented in this thesis in relation to the study hypotheses.

$H0_1$: There is no difference in reproducibility of measurement of lung diffusing capacity between EP-born and term-born subjects.

The hypothesis was sustained, as reproducibility of SB-DL_{CO} was similar between EP-born subjects and term-born controls, as well as between children and healthy adults, represented by the control group from the 1982–5 birth cohort.

$H0_2$: There are no differences in parameters of lung diffusing capacity obtained with the SB technique compared to the IB method

The hypothesis was rejected, as measurements of IB-DL_{CO} were significantly lower, compared to SB-DL_{CO}.

$H0_3$: There are no differences in lung diffusing capacity measurements between children, adolescents, and young adults born EP versus control subjects born at term.

The hypothesis was rejected, as significant deficits in DL_{CO} were detected between EP-born subjects and their matched term-born controls in both birth cohorts at both examinations.

$H0_4$: There are no differences in development of lung diffusing capacity and its subcomponents between EP-born subjects and term-born controls, from mid-childhood to adolescence and from adolescence to young adulthood.

The hypothesis was sustained, as no difference were found for DL_{CO} tracking between EP-born subjects and term-born controls from mid-childhood to young adulthood.

$H0_5$: There are no differences in lung diffusing capacity and cardiopulmonary outcomes between children born EP with PV-PPROM versus matched preterm-born control subjects with no PV-PPROM.

Some of the pulmonary function measurements (*z*-scores for FEV_1, FEV/FVC, and $FEF_{25–75}$) were significantly reduced in the PV-PPROM group, compared to preterm controls, whereas other measurements (lung volumes and diffusing capacity) were similar between both groups. Echocardiographic assessments revealed significant differences for TR velocity in the PV-PPROM group, compared to controls, indicating mild PH in the PV-PPROM group. Therefore, the first part of the hypothesis regarding lung diffusing capacity was partly rejected and partly sustained. The second part of the hypothesis regarding cardiopulmonary outcomes in relation to PH was rejected.

9 Future perspectives

Given the burden of an infant's birth at a time when their lung development is far from complete, and with most of the third trimester of pregnancy spent under NICU care further affecting their normal alveolar development, study results showing relatively modest decreases observed for lung diffusing capacity in the majority of long-term survivors of EP birth and PV-PPROM are intriguing. The finding of similar developmental patterns in EP- and term-born participants in two different birth cohorts from mid-childhood to early adulthood for measures of lung diffusing capacity is encouraging.

As the first large cohorts of EP-born subjects are now approaching their forties, their lung health through middle age is not yet mapped. There are abundant data to argue that airway obstruction tracks at a reduced level from EP birth through early childhood to adulthood, and that few of these individuals reach their expected peak FEV_1.[199, 338] Findings from the studies presented in this thesis indicate similar tracking also for DL_{CO}. Even though between-group differences were relatively small, the long-term consequences of generally lower DL_{CO} in EP-born subjects, compared to healthy controls, as they enter middle age, when decline of DL_{CO} normally starts, are not yet known.[245, 247] As one fears that EP-born subjects are at risk of developing COPD as adults,[215] and reduced DL_{CO} is a prognostic marker in COPD patients,[276, 339] in addition to being a predictor of all-cause mortality in COPD patients [340], and even in the absence of apparent clinical respiratory disease,[341] the study findings here indicate that measurements of DL_{CO} in EP-born subjects may be clinically relevant at all ages. A life-long obligation to ensure proper follow-up, treatment, and guidance fall upon the health profession that once made survival of these young individuals possible, and the study findings here suggest that DL_{CO} should be included in the follow-up programmes following EP birth. Moreover, the findings also underscore the fact that premature birth should be a focus not only for paediatricians, but also for general practitioners and adult pulmonologists.

The PV-PPROM study presented in Paper II was small, and data must therefore be interpreted with caution. However, its overall findings are encouraging. Studies of long-term consequences following PV-PPROM are lacking, and further and larger

studies are required to further our understanding of the impact on cardiopulmonary health. While the finding of mild PH in the PV-PPROM group is interesting—and needs to be confirmed by other studies, it also indicates that assessment for PH may be important in these individuals in the future.

Future studies of long-term pulmonary health following EP birth and PV-PPROM should preferably have a prospective design, including children from birth and optimally following up these children throughout childhood and adolescence and into adulthood. Larger studies that include more participants are preferable, as low statistical power often is an issue in these types of studies. However, the most important focus for researchers and health care workers within this field of medicine should be on preventing preterm birth itself, as well as on finding treatments that prevent the development of BPD and other respiratory impairments following preterm birth.

References

1. Eds CP Howson MK JLWHO. Born Too Soon: The Global action report on preterm birth. Geneva.: March of Dimes, PMNCH, Save the Children WHO. ; 2012.
2. Battaglia FC, Lubchenco LO. A practical classification of newborn infants by weight and gestational age. J Pediatr. 1967;71(2):159-63.
3. Status WECoP. The Use and Interpretation of Anthropometry Physical Status: the use and Interpretation of anthropometry World Health Organization; 1995.
4. Beune IM, Bloomfield FH, Ganzevoort W, Embleton ND, Rozance PJ, van Wassenaer-Leemhuis AG, et al. Consensus Based Definition of Growth Restriction in the Newborn. J Pediatr. 2018;196:71-6 e1.
5. Garite TJ, Clark R, Thorp JA. Intrauterine growth restriction increases morbidity and mortality among premature neonates. Am J Obstet Gynecol. 2004;191(2):481-7.
6. Zeitlin J, El Ayoubi M, Jarreau PH, Draper ES, Blondel B, Kunzel W, et al. Impact of fetal growth restriction on mortality and morbidity in a very preterm birth cohort. J Pediatr. 2010;157(5):733-9 e1.
7. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 2019;7(1):e37-e46.
8. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379(9832):2162-72.
9. Collaborators GBDMM. Global, regional, and national levels of maternal mortality, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1775-812.
10. (NIPH) NIoPH. Medical Birth Registry of Norway (MBRN) http://statistikkbank.fhi.no/mfr/2020 [
11. Stensvold HJ, Klingenberg C, Stoen R, Moster D, Braekke K, Guthe HJ, et al. Neonatal Morbidity and 1-Year Survival of Extremely Preterm Infants. Pediatrics. 2017;139(3).
12. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, et al. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. JAMA. 2015;314(10):1039-51.
13. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ. 2012;345:e7976.
14. Shah PS, Sankaran K, Aziz K, Allen AC, Seshia M, Ohlsson A, et al. Outcomes of preterm infants <29 weeks gestation over 10-year period in Canada: a cause for concern? J Perinatol. 2012;32(2):132-8.
15. Group E, Fellman V, Hellstrom-Westas L, Norman M, Westgren M, Kallen K, et al. One-year survival of extremely preterm infants after active perinatal care in Sweden. JAMA. 2009;301(21):2225-33.
16. Norman M, Hallberg B, Abrahamsson T, Bjorklund LJ, Domellof M, Farooqi A, et al. Association Between Year of Birth and 1-Year Survival Among Extremely

Preterm Infants in Sweden During 2004-2007 and 2014-2016. JAMA. 2019;321(12):1188-99.
17. Di Renzo GC, Cabero Roura L, Facchinetti F, Helmer H, Hubinont C, Jacobsson B, et al. Preterm Labor and Birth Management: Recommendations from the European Association of Perinatal Medicine. J Matern Fetal Neonatal Med. 2017;30(17):2011-30.
18. Cobo T, Kacerovsky M, Jacobsson B. Risk factors for spontaneous preterm delivery. Int J Gynaecol Obstet. 2020;150(1):17-23.
19. Ferrero DM, Larson J, Jacobsson B, Di Renzo GC, Norman JE, Martin JN, Jr., et al. Cross-Country Individual Participant Analysis of 4.1 Million Singleton Births in 5 Countries with Very High Human Development Index Confirms Known Associations but Provides No Biologic Explanation for 2/3 of All Preterm Births. PLoS One. 2016;11(9):e0162506.
20. Muglia LJ, Katz M. The enigma of spontaneous preterm birth. N Engl J Med. 2010;362(6):529-35.
21. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.
22. Cnattingius S. The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. Nicotine Tob Res. 2004;6 Suppl 2:S125-40.
23. Galinsky R, Polglase GR, Hooper SB, Black MJ, Moss TJ. The consequences of chorioamnionitis: preterm birth and effects on development. J Pregnancy. 2013;2013:412831.
24. Kozuki N, Lee AC, Silveira MF, Sania A, Vogel JP, Adair L, et al. The associations of parity and maternal age with small-for-gestational-age, preterm, and neonatal and infant mortality: a meta-analysis. BMC Public Health. 2013;13 Suppl 3:S2.
25. Poulsen G, Strandberg-Larsen K, Mortensen L, Barros H, Cordier S, Correia S, et al. Exploring educational disparities in risk of preterm delivery: a comparative study of 12 European birth cohorts. Paediatr Perinat Epidemiol. 2015;29(3):172-83.
26. Refuerzo JS. Impact of multiple births on late and moderate prematurity. Semin Fetal Neonatal Med. 2012;17(3):143-5.
27. Grimes-Dennis J, Berghella V. Cervical length and prediction of preterm delivery. Curr Opin Obstet Gynecol. 2007;19(2):191-5.
28. Brown WR. Association of preterm birth with brain malformations. Pediatr Res. 2009;65(6):642-6.
29. Mercer BM. Preterm premature rupture of the membranes. Obstet Gynecol. 2003;101(1):178-93.
30. Waters TP, Mercer BM. The management of preterm premature rupture of the membranes near the limit of fetal viability. Am J Obstet Gynecol. 2009;201(3):230-40.
31. Wagner P, Sonek J, Mayr S, Abele H, Goelz R, Hoopmann M, et al. Outcome of pregnancies with spontaneous PPROM before 24+0 weeks' gestation. Eur J Obstet Gynecol Reprod Biol. 2016;203:121-6.
32. Thomson AJ, Royal College of O, Gynaecologists. Care of Women Presenting with Suspected Preterm Prelabour Rupture of Membranes from 24(+0) Weeks of Gestation: Green-top Guideline No. 73. BJOG. 2019;126(9):e152-e66.

33. Veileder i fødselshjelp. Preterm vannavgang (pPROM) og primær vannavgang ved/nær termin (PROM): Norsk Gynekologisk Forening; 2020.
34. Menon R, Fortunato SJ. Infection and the role of inflammation in preterm premature rupture of the membranes. Best Pract Res Clin Obstet Gynaecol. 2007;21(3):467-78.
35. Oh KJ, Romero R, Park JY, Hong JS, Yoon BH. The earlier the gestational age, the greater the intensity of the intra-amniotic inflammatory response in women with preterm premature rupture of membranes and amniotic fluid infection by Ureaplasma species. J Perinat Med. 2019;47(5):516-27.
36. Menon R, Richardson LS. Preterm prelabor rupture of the membranes: A disease of the fetal membranes. Semin Perinatol. 2017;41(7):409-19.
37. Apgar V, Holaday DA, James LS, Weisbrot IM, Berrien C. Evaluation of the newborn infant; second report. J Am Med Assoc. 1958;168(15):1985-8.
38. Philip AG. The evolution of neonatology. Pediatr Res. 2005;58(4):799-815.
39. Owen LS, Manley BJ, Davis PG, Doyle LW. The evolution of modern respiratory care for preterm infants. Lancet. 2017;389(10079):1649-59.
40. Askie LM, Darlow BA, Davis PG, Finer N, Stenson B, Vento M, et al. Effects of targeting lower versus higher arterial oxygen saturations on death or disability in preterm infants. Cochrane Database Syst Rev. 2017;4:CD011190.
41. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics. 1972;50(4):515-25.
42. Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2017;3:CD004454.
43. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. AMA J Dis Child. 1959;97(5, Part 1):517-23.
44. Fujiwara T, Maeta H, Chida S, Morita T, Watabe Y, Abe T. Artificial surfactant therapy in hyaline-membrane disease. Lancet. 1980;1(8159):55-9.
45. Jobe AH. Pulmonary surfactant therapy. N Engl J Med. 1993;328(12):861-8.
46. Starr SE. Antimicrobial therapy of bacterial sepsis in the newborn infant. J Pediatr. 1985;106(6):1043-8.
47. Pereira GR, Barbosa NM. Controversies in neonatal nutrition. Pediatr Clin North Am. 1986;33(1):65-89.
48. McNelis K, Fu TT, Poindexter B. Nutrition for the Extremely Preterm Infant. Clin Perinatol. 2017;44(2):395-406.
49. Brown ER. Increased risk of bronchopulmonary dysplasia in infants with patent ductus arteriosus. J Pediatr. 1979;95(5 Pt 2):865-6.
50. Su BH, Lin HY, Chiu HY, Tsai ML, Chen YT, Lu IC. Therapeutic strategy of patent ductus arteriosus in extremely preterm infants. Pediatr Neonatol. 2020;61(2):133-41.
51. Boundy EO, Dastjerdi R, Spiegelman D, Fawzi WW, Missmer SA, Lieberman E, et al. Kangaroo Mother Care and Neonatal Outcomes: A Meta-analysis. Pediatrics. 2016;137(1).

52. Charpak N, Tessier R, Ruiz JG, Hernandez JT, Uriza F, Villegas J, et al. Twenty-year Follow-up of Kangaroo Mother Care Versus Traditional Care. Pediatrics. 2017;139(1).
53. Ortenstrand A, Westrup B, Brostrom EB, Sarman I, Akerstrom S, Brune T, et al. The Stockholm Neonatal Family Centered Care Study: effects on length of stay and infant morbidity. Pediatrics. 2010;125(2):e278-85.
54. White RD. The newborn intensive care unit environment of care: how we got here, where we're headed, and why. Semin Perinatol. 2011;35(1):2-7.
55. Guillen U, Weiss EM, Munson D, Maton P, Jefferies A, Norman M, et al. Guidelines for the Management of Extremely Premature Deliveries: A Systematic Review. Pediatrics. 2015;136(2):343-50.
56. Singh J, Fanaroff J, Andrews B, Caldarelli L, Lagatta J, Plesha-Troyke S, et al. Resuscitation in the "gray zone" of viability: determining physician preferences and predicting infant outcomes. Pediatrics. 2007;120(3):519-26.
57. Wilkinson D, Hayden D. In Search of Consistency: Scandinavian Approaches to Resuscitation of Extremely Preterm Infants. Pediatrics. 2018;142(Suppl 1):S603-S6.
58. Syltern J, Markestad T, Saugstad OD, Stoen R. NICU Dialects: Understanding Norwegian Practice Variation. Pediatrics. 2018;142(Suppl 1):S545-S51.
59. Domellof M, Pettersson K. [Guidelines for threatening premature birth will provide better and more equal care]. Lakartidningen. 2017;114.
60. Backes CH, Rivera BK, Pavlek L, Beer LJ, Ball MK, Zettler ET, et al. Proactive neonatal treatment at 22 weeks of gestation: a systematic review and meta-analysis. Am J Obstet Gynecol. 2021;224(2):158-74.
61. Rysavy MA, Mehler K, Oberthur A, Agren J, Kusuda S, McNamara PJ, et al. An Immature Science: Intensive Care for Infants Born at </=23 Weeks of Gestation. J Pediatr. 2021;233:16-25 e1.
62. Smith LJ, McKay KO, van Asperen PP, Selvadurai H, Fitzgerald DA. Normal development of the lung and premature birth. Paediatr Respir Rev. 2010;11(3):135-42.
63. Stocks J, Hislop A, Sonnappa S. Early lung development: lifelong effect on respiratory health and disease. Lancet Respir Med. 2013;1(9):728-42.
64. Mullassery D, Smith NP. Lung development. Semin Pediatr Surg. 2015;24(4):152-5.
65. Sardesai S, Biniwale M, Wertheimer F, Garingo A, Ramanathan R. Evolution of surfactant therapy for respiratory distress syndrome: past, present, and future. Pediatr Res. 2017;81(1-2):240-8.
66. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Plavka R, et al. European Consensus Guidelines on the Management of Respiratory Distress Syndrome - 2016 Update. Neonatology. 2017;111(2):107-25.
67. Hislop AA. Airway and blood vessel interaction during lung development. J Anat. 2002;201(4):325-34.
68. Narayanan M, Beardsmore CS, Owers-Bradley J, Dogaru CM, Mada M, Ball I, et al. Catch-up alveolarization in ex-preterm children: evidence from (3)He magnetic resonance. Am J Respir Crit Care Med. 2013;187(10):1104-9.
69. Narayanan M, Owers-Bradley J, Beardsmore CS, Mada M, Ball I, Garipov R, et al. Alveolarization continues during childhood and adolescence: new evidence from helium-3 magnetic resonance. Am J Respir Crit Care Med. 2012;185(2):186-91.

70. Quanjer PH, Stanojevic S, Stocks J, Hall GL, Prasad KV, Cole TJ, et al. Changes in the FEV(1)/FVC ratio during childhood and adolescence: an intercontinental study. Eur Respir J. 2010;36(6):1391-9.
71. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-43.
72. Boezen HM, Jansen DF, Postma DS. Sex and gender differences in lung development and their clinical significance. Clin Chest Med. 2004;25(2):237-45.
73. Gibbons JTD, Wilson AC, Simpson SJ. Predicting Lung Health Trajectories for Survivors of Preterm Birth. Front Pediatr. 2020;8:318.
74. Agusti A, Faner R. Lung function trajectories in health and disease. Lancet Respir Med. 2019;7(4):358-64.
75. Bui DS, Lodge CJ, Burgess JA, Lowe AJ, Perret J, Bui MQ, et al. Childhood predictors of lung function trajectories and future COPD risk: a prospective cohort study from the first to the sixth decade of life. Lancet Respir Med. 2018;6(7):535-44.
76. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010;126(3):443-56.
77. Anadkat JS, Kuzniewicz MW, Chaudhari BP, Cole FS, Hamvas A. Increased risk for respiratory distress among white, male, late preterm and term infants. J Perinatol. 2012;32(10):780-5.
78. Curtis P. Hyaline membrane disease. J Pediatr. 1957;51(6):726-41.
79. McPherson C, Wambach JA. Prevention and Treatment of Respiratory Distress Syndrome in Preterm Neonates. Neonatal Netw. 2018;37(3):169-77.
80. Edwards MO, Kotecha SJ, Kotecha S. Respiratory distress of the term newborn infant. Paediatr Respir Rev. 2013;14(1):29-36; quiz -7.
81. Kallem VR, Pandita A, Pillai A. Infant of diabetic mother: what one needs to know? J Matern Fetal Neonatal Med. 2020;33(3):482-92.
82. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Te Pas A, et al. European Consensus Guidelines on the Management of Respiratory Distress Syndrome - 2019 Update. Neonatology. 2019;115(4):432-50.
83. Northway WH, Jr., Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. N Engl J Med. 1967;276(7):357-68.
84. Bonikos DS, Bensch KG, Northway WH, Jr., Edwards DK. Bronchopulmonary dysplasia: the pulmonary pathologic sequel of necrotizing bronchiolitis and pulmonary fibrosis. Hum Pathol. 1976;7(6):643-66.
85. Baraldi E, Filippone M. Chronic lung disease after premature birth. N Engl J Med. 2007;357(19):1946-55.
86. Jobe AJ. The new BPD: an arrest of lung development. Pediatr Res. 1999;46(6):641-3.
87. Thebaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. Nat Rev Dis Primers. 2019;5(1):78.
88. Kim SR, Kim DI, Kim SH, Lee H, Lee KS, Cho SH, et al. NLRP3 inflammasome activation by mitochondrial ROS in bronchial epithelial cells is required for allergic inflammation. Cell Death Dis. 2014;5:e1498.

89. Bhandari V. Postnatal inflammation in the pathogenesis of bronchopulmonary dysplasia. Birth Defects Res A Clin Mol Teratol. 2014;100(3):189-201.
90. Koksal N, Kayik B, Cetinkaya M, Ozkan H, Budak F, Kilic S, et al. Value of serum and bronchoalveolar fluid lavage pro- and anti-inflammatory cytokine levels for predicting bronchopulmonary dysplasia in premature infants. Eur Cytokine Netw. 2012;23(2):29-35.
91. Barazzone C, White CW. Mechanisms of cell injury and death in hyperoxia: role of cytokines and Bcl-2 family proteins. Am J Respir Cell Mol Biol. 2000;22(5):517-9.
92. Walsh MC, Szefler S, Davis J, Allen M, Van Marter L, Abman S, et al. Summary proceedings from the bronchopulmonary dysplasia group. Pediatrics. 2006;117(3 Pt 2):S52-6.
93. Zeitlin J, Draper ES, Kollee L, Milligan D, Boerch K, Agostino R, et al. Differences in rates and short-term outcome of live births before 32 weeks of gestation in Europe in 2003: results from the MOSAIC cohort. Pediatrics. 2008;121(4):e936-44.
94. Isayama T, Lee SK, Mori R, Kusuda S, Fujimura M, Ye XY, et al. Comparison of mortality and morbidity of very low birth weight infants between Canada and Japan. Pediatrics. 2012;130(4):e957-65.
95. Markestad T, Kaaresen PI, Ronnestad A, Reigstad H, Lossius K, Medbo S, et al. Early death, morbidity, and need of treatment among extremely premature infants. Pediatrics. 2005;115(5):1289-98.
96. Hwang JS, Rehan VK. Recent Advances in Bronchopulmonary Dysplasia: Pathophysiology, Prevention, and Treatment. Lung. 2018;196(2):129-38.
97. Bonadies L, Zaramella P, Porzionato A, Perilongo G, Muraca M, Baraldi E. Present and Future of Bronchopulmonary Dysplasia. J Clin Med. 2020;9(5).
98. Philpot PA, Bhandari V. Predicting the likelihood of bronchopulmonary dysplasia in premature neonates. Expert Rev Respir Med. 2019;13(9):871-84.
99. Jobe AH, Bancalari E. An All-Inclusive Perspective on Bronchopulmonary Dysplasia. J Pediatr. 2021.
100. Lal CV, Bhandari V, Ambalavanan N. Genomics, microbiomics, proteomics, and metabolomics in bronchopulmonary dysplasia. Semin Perinatol. 2018;42(7):425-31.
101. Bentsen MH, Markestad T, Halvorsen T. Ventilator flow data predict bronchopulmonary dysplasia in extremely premature neonates. ERJ Open Res. 2018;4(1).
102. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;163(7):1723-9.
103. Poindexter BB, Feng R, Schmidt B, Aschner JL, Ballard RA, Hamvas A, et al. Comparisons and Limitations of Current Definitions of Bronchopulmonary Dysplasia for the Prematurity and Respiratory Outcomes Program. Ann Am Thorac Soc. 2015;12(12):1822-30.
104. Higgins RD, Jobe AH, Koso-Thomas M, Bancalari E, Viscardi RM, Hartert TV, et al. Bronchopulmonary Dysplasia: Executive Summary of a Workshop. J Pediatr. 2018;197:300-8.

105. Jensen EA, Dysart K, Gantz MG, McDonald S, Bamat NA, Keszler M, et al. The Diagnosis of Bronchopulmonary Dysplasia in Very Preterm Infants. An Evidence-based Approach. Am J Respir Crit Care Med. 2019;200(6):751-9.
106. Keller RL, Feng R, DeMauro SB, Ferkol T, Hardie W, Rogers EE, et al. Bronchopulmonary Dysplasia and Perinatal Characteristics Predict 1-Year Respiratory Outcomes in Newborns Born at Extremely Low Gestational Age: A Prospective Cohort Study. J Pediatr. 2017;187:89-97 e3.
107. Effect of corticosteroids for fetal maturation on perinatal outcomes. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. JAMA. 1995;273(5):413-8.
108. Committee on F, Newborn, American Academy of P. Respiratory support in preterm infants at birth. Pediatrics. 2014;133(1):171-4.
109. Fischer HS, Buhrer C. Avoiding endotracheal ventilation to prevent bronchopulmonary dysplasia: a meta-analysis. Pediatrics. 2013;132(5):e1351-60.
110. Lemyre B, Laughon M, Bose C, Davis PG. Early nasal intermittent positive pressure ventilation (NIPPV) versus early nasal continuous positive airway pressure (NCPAP) for preterm infants. Cochrane Database Syst Rev. 2016;12:CD005384.
111. Shi Y, Muniraman H, Biniwale M, Ramanathan R. A Review on Non-invasive Respiratory Support for Management of Respiratory Distress in Extremely Preterm Infants. Front Pediatr. 2020;8:270.
112. Seger N, Soll R. Animal derived surfactant extract for treatment of respiratory distress syndrome. Cochrane Database Syst Rev. 2009(2):CD007836.
113. Soll RF. Synthetic surfactant for respiratory distress syndrome in preterm infants. Cochrane Database Syst Rev. 2000(2):CD001149.
114. Isayama T, Iwami H, McDonald S, Beyene J. Association of Noninvasive Ventilation Strategies With Mortality and Bronchopulmonary Dysplasia Among Preterm Infants: A Systematic Review and Meta-analysis. JAMA. 2016;316(6):611-24.
115. Arattu Thodika FMS, Ambulkar H, Williams E, Bhat R, Dassios T, Greenough A. Outcomes following less-invasive-surfactant-administration in the delivery-room. Early Hum Dev. 2022;167:105562.
116. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110(2 Pt 1):285-91.
117. Doyle LW, Ehrenkranz RA, Halliday HL. Early (< 8 days) postnatal corticosteroids for preventing chronic lung disease in preterm infants. Cochrane Database Syst Rev. 2014(5):CD001146.
118. Doyle LW, Halliday HL, Ehrenkranz RA, Davis PG, Sinclair JC. Impact of postnatal systemic corticosteroids on mortality and cerebral palsy in preterm infants: effect modification by risk for chronic lung disease. Pediatrics. 2005;115(3):655-61.
119. Doyle LW, Halliday HL, Ehrenkranz RA, Davis PG, Sinclair JC. An update on the impact of postnatal systemic corticosteroids on mortality and cerebral palsy in preterm infants: effect modification by risk of bronchopulmonary dysplasia. J Pediatr. 2014;165(6):1258-60.

120. Doyle LW, Ehrenkranz RA, Halliday HL. Dexamethasone treatment after the first week of life for bronchopulmonary dysplasia in preterm infants: a systematic review. Neonatology. 2010;98(4):289-96.
121. Baud O, Maury L, Lebail F, Ramful D, El Moussawi F, Nicaise C, et al. Effect of early low-dose hydrocortisone on survival without bronchopulmonary dysplasia in extremely preterm infants (PREMILOC): a double-blind, placebo-controlled, multicentre, randomised trial. Lancet. 2016;387(10030):1827-36.
122. Bassler D, Plavka R, Shinwell ES, Hallman M, Jarreau PH, Carnielli V, et al. Early Inhaled Budesonide for the Prevention of Bronchopulmonary Dysplasia. N Engl J Med. 2015;373(16):1497-506.
123. Heo M, Jeon GW. Intratracheal administration of budesonide with surfactant in very low birth weight infants to prevent bronchopulmonary dysplasia. Turk J Pediatr. 2020;62(4):551-9.
124. Papagianis PC, Pillow JJ, Moss TJ. Bronchopulmonary dysplasia: Pathophysiology and potential anti-inflammatory therapies. Paediatr Respir Rev. 2019;30:34-41.
125. Lignelli E, Palumbo F, Myti D, Morty RE. Recent advances in our understanding of the mechanisms of lung alveolarization and bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol. 2019;317(6):L832-L87.
126. Sim WH, Ng H, Sheehan P. Maternal and neonatal outcomes following expectant management of preterm prelabor rupture of membranes before viability. J Matern Fetal Neonatal Med. 2020;33(4):533-41.
127. Manoj MG, Kakkar S. Potter's syndrome - a fatal constellation of anomalies. Indian J Med Res. 2014;139(4):648-9.
128. Beydoun SN, Yasin SY. Premature rupture of the membranes before 28 weeks: conservative management. Am J Obstet Gynecol. 1986;155(3):471-9.
129. LeMoine F, Moore RC, Chapple A, Moore FA, Sutton E. Neonatal Survivability following Previable PPROM after Hospital Readmission for Intervention. AJP Rep. 2020;10(4):e395-e402.
130. Sim WH, Araujo Junior E, Da Silva Costa F, Sheehan PM. Maternal and neonatal outcomes following expectant management of preterm prelabour rupture of membranes before viability. J Perinat Med. 2017;45(1):29-44.
131. Kibel M, Asztalos E, Barrett J, Dunn MS, Tward C, Pittini A, et al. Outcomes of Pregnancies Complicated by Preterm Premature Rupture of Membranes Between 20 and 24 Weeks of Gestation. Obstet Gynecol. 2016;128(2):313-20.
132. Manuck TA, Varner MW. Neonatal and early childhood outcomes following early vs later preterm premature rupture of membranes. Am J Obstet Gynecol. 2014;211(3):308 e1-6.
133. Acaia B, Crovetto F, Ossola MW, Nozza S, Baffero GM, Somigliana E, et al. Predictive factors for neonatal survival in women with periviable preterm rupture of the membranes. J Matern Fetal Neonatal Med. 2013;26(16):1628-34.
134. Chauleur C, Rochigneux S, Seffert P, Chene G, Billiemaz K, Collet F. Neonatal outcomes and four-year follow-up after spontaneous or iatrogenic preterm prelabor rupture of membranes before 24 weeks. Acta Obstet Gynecol Scand. 2009;88(7):801-6.

135. Pristauz G, Bauer M, Maurer-Fellbaum U, Rotky-Fast C, Bader AA, Haas J, et al. Neonatal outcome and two-year follow-up after expectant management of second trimester rupture of membranes. Int J Gynaecol Obstet. 2008;101(3):264-8.
136. Steinhorn RH. Neonatal pulmonary hypertension. Pediatr Crit Care Med. 2010;11(2 Suppl):S79-84.
137. Bui CB, Pang MA, Sehgal A, Theda C, Lao JC, Berger PJ, et al. Pulmonary hypertension associated with bronchopulmonary dysplasia in preterm infants. J Reprod Immunol. 2017;124:21-9.
138. Rosenzweig EB, Abman SH, Adatia I, Beghetti M, Bonnet D, Haworth S, et al. Paediatric pulmonary arterial hypertension: updates on definition, classification, diagnostics and management. Eur Respir J. 2019;53(1).
139. Teng RJ, Wu TJ. Persistent pulmonary hypertension of the newborn. J Formos Med Assoc. 2013;112(4):177-84.
140. O'Connor MG, Cornfield DN, Austin ED. Pulmonary hypertension in the premature infant: a challenging comorbidity in a vulnerable population. Curr Opin Pediatr. 2016;28(3):324-30.
141. Abman SH, Hansmann G, Archer SL, Ivy DD, Adatia I, Chung WK, et al. Pediatric Pulmonary Hypertension: Guidelines From the American Heart Association and American Thoracic Society. Circulation. 2015;132(21):2037-99.
142. Arjaans S, Zwart EAH, Ploegstra MJ, Bos AF, Kooi EMW, Hillege HL, et al. Identification of gaps in the current knowledge on pulmonary hypertension in extremely preterm infants: A systematic review and meta-analysis. Paediatr Perinat Epidemiol. 2018;32(3):258-67.
143. Naumburg E, Söderström L. Increased risk of pulmonary hypertension following premature birth. BMC Pediatr. 2019;19(1):288-.
144. Baker CD, Abman SH, Mourani PM. Pulmonary Hypertension in Preterm Infants with Bronchopulmonary Dysplasia. Pediatr Allergy Immunol Pulmonol. 2014;27(1):8-16.
145. Altit G, Dancea A, Renaud C, Perreault T, Lands LC, Sant'Anna G. Pathophysiology, screening and diagnosis of pulmonary hypertension in infants with bronchopulmonary dysplasia - A review of the literature. Paediatr Respir Rev. 2017;23:16-26.
146. Kumar VHS. Diagnostic Approach to Pulmonary Hypertension in Premature Neonates. Children (Basel). 2017;4(9):75.
147. Williams O, Hutchings G, Hubinont C, Debauche C, Greenough A. Pulmonary effects of prolonged oligohydramnios following mid-trimester rupture of the membranes--antenatal and postnatal management. Neonatology. 2012;101(2):83-90.
148. Aikio O, Metsola J, Vuolteenaho R, Perhomaa M, Hallman M. Transient defect in nitric oxide generation after rupture of fetal membranes and responsiveness to inhaled nitric oxide in very preterm infants with hypoxic respiratory failure. J Pediatr. 2012;161(3):397-403.e1.
149. Kwan WC, Shavelle DM, Laughrun DR. Pulmonary vascular resistance index: Getting the units right and why it matters. Clin Cardiol. 2019;42(3):334-8.
150. Nord-Norge B-ouU. Persisterende pulmonal hypertensjon hos nyfødte. Metodebok i nyfødtmedisin. 6 ed. Tromsø.2019. p. 129-32.

151. Stocker JT. Pathologic features of long-standing "healed" bronchopulmonary dysplasia: a study of 28 3- to 40-month-old infants. Hum Pathol. 1986;17(9):943-61.
152. Sobonya RE, Logvinoff MM, Taussig LM, Theriault A. Morphometric analysis of the lung in prolonged bronchopulmonary dysplasia. Pediatr Res. 1982;16(11):969-72.
153. Hislop AA, Wigglesworth JS, Desai R, Aber V. The effects of preterm delivery and mechanical ventilation on human lung growth. Early Hum Dev. 1987;15(3):147-64.
154. Margraf LR, Tomashefski JF, Jr., Bruce MC, Dahms BB. Morphometric analysis of the lung in bronchopulmonary dysplasia. Am Rev Respir Dis. 1991;143(2):391-400.
155. Van Lierde S, Cornelis A, Devlieger H, Moerman P, Lauweryns J, Eggermont E. Different patterns of pulmonary sequelae after hyaline membrane disease: heterogeneity of bronchopulmonary dysplasia? A clinicopathologic study. Biol Neonate. 1991;60(3-4):152-62.
156. Chambers HM, van Velzen D. Ventilator-related pathology in the extremely immature lung. Pathology. 1989;21(2):79-83.
157. Husain AN, Siddiqui NH, Stocker JT. Pathology of arrested acinar development in postsurfactant bronchopulmonary dysplasia. Hum Pathol. 1998;29(7):710-7.
158. Cutz E, Chiasson D. Chronic lung disease after premature birth. N Engl J Med. 2008;358(7):743-5; author reply 5-6.
159. Galderisi A, Calabrese F, Fortarezza F, Abman S, Baraldi E. Airway Histopathology of Adolescent Survivors of Bronchopulmonary Dysplasia. J Pediatr. 2019;211:215-8.
160. Liu N, Cummings OW, Lagstein A, Hage CA, Chan KM, Zhang C. Lung Transplantation for Bronchopulmonary Dysplasia in Adults: A Clinical and Pathologic Study of 3 Cases. Am J Surg Pathol. 2020;44(4):509-15.
161. Lee YP, Chun EM, Kim YK, Sung SH. Two Cases of Bronchopulmonary Dysplasia of Similar Appearance in Adult Monozygotic Twin: Pathology and Computed Tomographic Findings. Tuberc Respir Dis. 2015;78(2):128-32.
162. Bland RD, Coalson JJ. Chronic Lung Disease in Early Infancy. Baton Rouge, UNITED STATES: Taylor & Francis Group; 1999.
163. Coalson JJ. Pathology of bronchopulmonary dysplasia. Semin Perinatol. 2006;30(4):179-84.
164. Coalson JJ. Pathology of new bronchopulmonary dysplasia. Semin Neonatol. 2003;8(1):73-81.
165. Bhatt AJ, Pryhuber GS, Huyck H, Watkins RH, Metlay LA, Maniscalco WM. Disrupted pulmonary vasculature and decreased vascular endothelial growth factor, Flt-1, and TIE-2 in human infants dying with bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;164(10 Pt 1):1971-80.
166. Thibeault DW, Mabry SM, Norberg M, Truog WE, Elekezie II. Lung microvascular adaptation in infants with chronic lung disease. Biology of the Neonate. 2004;85(4):273-82.
167. Berger J, Bhandari V. Animal models of bronchopulmonary dysplasia. The term mouse models. 2014;307(12):L936-47.

168. O'Reilly M, Thebaud B. Animal models of bronchopulmonary dysplasia. The term rat models. 2014;307(12):L948-58.
169. D'Angio CT, Ryan RM. Animal models of bronchopulmonary dysplasia. The preterm and term rabbit models. 2014;307(12):L959-69.
170. Albertine KH. Utility of large-animal models of BPD: chronically ventilated preterm lambs. 2015;308(10):L983-L1001.
171. Dargaville PA, Lavizzari A, Padoin P, Black D, Zonneveld E, Perkins E, et al. An authentic animal model of the very preterm infant on nasal continuous positive airway pressure. Intensive Care Med Exp. 2015;3(1):51.
172. Caminita F, van der Merwe M, Hance B, Krishnan R, Miller S, Buddington K, et al. A preterm pig model of lung immaturity and spontaneous infant respiratory distress syndrome. 2015;308(2):L118-29.
173. Yoder BA, Coalson JJ. Animal models of bronchopulmonary dysplasia. The preterm baboon models. 2014;307(12):L970-7.
174. Morty RE. Recent advances in the pathogenesis of BPD. Semin Perinatol. 2018;42(7):404-12.
175. Lamarche-Vadel A, Blondel B, Truffert P, Burguet A, Cambonie G, Selton D, et al. Re-hospitalization in infants younger than 29 weeks' gestation in the EPIPAGE cohort. Acta Paediatr. 2004;93(10):1340-5.
176. Skromme K, Leversen KT, Eide GE, Markestad T, Halvorsen T. Respiratory illness contributed significantly to morbidity in children born extremely premature or with extremely low birthweights in 1999-2000. Acta Paediatrica. 2015;104(11):1189-98.
177. Proietti E, Riedel T, Fuchs O, Pramana I, Singer F, Schmidt A, et al. Can infant lung function predict respiratory morbidity during the first year of life in preterm infants? Eur Respir J. 2014;43(6):1642-51.
178. Priante E, Moschino L, Mardegan V, Manzoni P, Salvadori S, Baraldi E. Respiratory Outcome after Preterm Birth: A Long and Difficult Journey. American Journal of Perinatology. 2016;33(11):1040-2.
179. Skromme K, Vollsaeter M, Oymar K, Markestad T, Halvorsen T. Respiratory morbidity through the first decade of life in a national cohort of children born extremely preterm. BMC Pediatr. 2018;18.
180. Mowitz ME, Ayyagari R, Gao W, Zhao J, Mangili A, Sarda SP. Health Care Burden of Bronchopulmonary Dysplasia Among Extremely Preterm Infants. Front Pediatr. 2019;7:510.
181. Doyle LW, Ford G, Davis N. Health and hospitalistions after discharge in extremely low birth weight infants. Semin Neonatol. 2003;8(2):137-45.
182. Cheong JLY, Doyle LW. An update on pulmonary and neurodevelopmental outcomes of bronchopulmonary dysplasia. Semin Perinatol. 2018;42(7):478-84.
183. Been JV, Lugtenberg MJ, Smets E, van Schayck CP, Kramer BW, Mommers M, et al. Preterm Birth and Childhood Wheezing Disorders: A Systematic Review and Meta-Analysis. PLoS Med. 2014;11(1).
184. Fawke J, Lum S, Kirkby J, Hennessy E, Marlow N, Rowell V, et al. Lung Function and Respiratory Symptoms at 11 Years in Children Born Extremely Preterm The EPICure Study. American Journal of Respiratory and Critical Care Medicine. 2010;182(2):237-45.

185. Voort AMMSVD, Arends LR, Jongste JC, Annesi-Maesano I, Arshad SH, Barros H, et al. Preterm birth, infant weight gain, and childhood asthma risk: A meta-analysis of 147,000 European children. J Allergy Clin Immunol. 2014;133(5):1317-29.
186. Vollsaeter M, Skromme K, Satrell E, Clemm H, Roksund O, Oymar K, et al. Children Born Preterm at the Turn of the Millennium Had Better Lung Function Than Children Born Similarly Preterm in the Early 1990s. PLoS One. 2015;10(12):e0144243.
187. Kotecha SJ, Edwards MO, Watkins WJ, Henderson AJ, Paranjothy S, Dunstan FD, et al. Effect of preterm birth on later FEV1: a systematic review and meta-analysis. Thorax. 2013;68(8):760-6.
188. Fortuna M, Carraro S, Temporin E, Berardi M, Zanconato S, Salvadori S, et al. Mid-childhood lung function in a cohort of children with "new bronchopulmonary dysplasia". Pediatr Pulmonol. 2016;51(10):1057-64.
189. Islam JY, Keller RL, Aschner JL, Hartert TV, Moore PE. Understanding the Short- and Long-Term Respiratory Outcomes of Prematurity and Bronchopulmonary Dysplasia. Am J Respir Crit Care Med. 2015;192(2):134-56.
190. Halvorsen T, Skadberg BT, Eide GE, Roksund OD, Markestad T. Better care of immature infants; has it influenced long-term pulmonary outcome? Acta Paediatr. 2006;95(5):547-54.
191. Ronkainen E, Perhomaa M, Mattila L, Hallman M, Dunder T. Structural Pulmonary Abnormalities Still Evident in Schoolchildren with New Bronchopulmonary Dysplasia. Neonatology. 2018;113(2):122-30.
192. Aukland SM, Halvorsen T, Fosse KR, Daltveit AK, Rosendahl K. High-resolution CT of the chest in children and young adults who were born prematurely: findings in a population-based study. AJR Am J Roentgenol. 2006;187(4):1012-8.
193. Vrijlandt EJLE, Gerritsen J, Boezen HM, Grevink RG, Duiverman EJ. Lung function and exercise capacity in young adults born prematurely. American Journal of Respiratory and Critical Care Medicine. 2006;173(8):890-6.
194. Halvorsen T, Skadberg BT, Eide GE, Roksund OD, Carlsen KH, Bakke P. Pulmonary outcome in adolescents of extreme preterm birth: a regional cohort study. Acta Paediatr. 2004;93(10):1294-300.
195. Doyle LW, Faber B, Callanan C, Freezer N, Ford GW, Davis NM. Bronchopulmonary dysplasia in very low birth weight subjects and lung function in late adolescence. Pediatrics. 2006;118(1):108-13.
196. Narang I, Bush A, Rosenthal M. Gas Transfer and Pulmonary Blood Flow at Rest and during Exercise in Adults 21 Years after Preterm Birth. American Journal of Respiratory and Critical Care Medicine. 2009;180(4):339-45.
197. Gough A, Spence D, Linden M, Halliday HL, McGarvey LPA. General and Respiratory Health Outcomes in Adult Survivors of Bronchopulmonary Dysplasia A Systematic Review. Chest. 2012;141(6):1554-67.
198. Wong PM, Lees AN, Louw J, Lee FY, French N, Gain K, et al. Emphysema in young adult survivors of moderate-to-severe bronchopulmonary dysplasia. European Respiratory Journal. 2008;32(2):321-8.
199. Vollsaeter M, Roksund OD, Eide GE, Markestad T, Halvorsen T. Lung function after preterm birth: development from mid-childhood to adulthood. Thorax. 2013;68(8):767-76.

200. Doyle LW, Adams AM, Robertson C, Ranganathan S, Davis NM, Lee KJ, et al. Increasing airway obstruction from 8 to 18 years in extremely preterm/low-birthweight survivors born in the surfactant era. Thorax. 2017;72(8):712-9.
201. Hurst JR, Beckmann J, Ni Y, Bolton CE, McEniery CM, Cockcroft JR, et al. Respiratory and Cardiovascular Outcomes in Survivors of Extremely Preterm Birth at 19 Years. Am J Respir Crit Care Med. 2020;202(3):422-32.
202. Um-Bergstrom P, Hallberg J, Thunqvist P, Berggren-Brostrom E, Anderson M, Adenfelt G, et al. Lung function development after preterm birth in relation to severity of Bronchopulmonary dysplasia. BMC Pulm Med. 2017;17(1):97.
203. Doyle LW, Carse E, Adams AM, Ranganathan S, Opie G, Cheong JLY, et al. Ventilation in Extremely Preterm Infants and Respiratory Function at 8 Years. N Engl J Med. 2017;377(4):329-37.
204. Howling SJ, Northway WH, Hansell DM, Moss RB, Ward S, Muller NL. Pulmonary sequelae of bronchopulmonary dysplasia survivors: High-resolution CT findings. American Journal of Roentgenology. 2000;174(5):1323-6.
205. Northway WH, Moss RB, Carlisle KB, Parker BR, Popp RL, Pitlick PT, et al. Late Pulmonary Sequelae of Bronchopulmonary Dysplasia. New England Journal of Medicine. 1990;323(26):1793-9.
206. Gough A, Linden M, Spence D, Patterson CC, Halliday HL, McGarvey LP. Impaired lung function and health status in adult survivors of bronchopulmonary dysplasia. Eur Respir J. 2014;43(3):808-16.
207. Vollsaeter M, Clemm HH, Satrell E, Eide GE, Roksund OD, Markestad T, et al. Adult Respiratory Outcomes of Extreme Preterm Birth A Regional Cohort Study. Annals of the American Thoracic Society. 2015;12(3):313-22.
208. Bardsen T, Roksund OD, Benestad MR, Hufthammer KO, Clemm HH, Mikalsen IB, et al. Tracking of lung function from 10 to 35 years after being born extremely preterm or with extremely low birth weight. Thorax. 2022;77(8):790-8.
209. Yang J, Kingsford RA, Horwood J, Epton MJ, Swanney MP, Stanton J, et al. Lung Function of Adults Born at Very Low Birth Weight. Pediatrics. 2020;145(2).
210. World Health Organization. The top 10 causes of death: WHO; 2020 [Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.
211. Diseases GBD, Injuries C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396(10258):1204-22.
212. Global Initiative for Chronic Obstructive Lung Disease. 2021 Global strategy for prevention, diagnosis and management of COPD Fontana, WI, USA2020 [Available from: www.goldcopd.org.
213. Postma DS, Bush A, van den Berge M. Risk factors and early origins of chronic obstructive pulmonary disease. Lancet. 2015;385(9971):899-909.
214. Savran O, Ulrik CS. Early life insults as determinants of chronic obstructive pulmonary disease in adult life. Int J Chron Obstruct Pulmon Dis. 2018;13:683-93.
215. McGrath-Morrow SA, Collaco JM. Bronchopulmonary dysplasia: what are its links to COPD? Ther Adv Respir Dis. 2019;13:1753466619892492.
216. Saad NJ, Patel J, Burney P, Minelli C. Birth Weight and Lung Function in Adulthood: A Systematic Review and Meta-analysis. Ann Am Thorac Soc. 2017;14(6):994-1004.

217. Lawlor DA, Ebrahim S, Davey Smith G. Association of birth weight with adult lung function: findings from the British Women's Heart and Health Study and a meta-analysis. Thorax. 2005;60(10):851-8.
218. Allinson JP, Hardy R, Donaldson GC, Shaheen SO, Kuh D, Wedzicha JA. Combined Impact of Smoking and Early-Life Exposures on Adult Lung Function Trajectories. Am J Respir Crit Care Med. 2017;196(8):1021-30.
219. Lopez-Campos JL, Soler-Cataluna JJ, Miravitlles M. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2019 Report: Future Challenges. Arch Bronconeumol. 2020;56(2):65-7.
220. Carraro S, Scheltema N, Bont L, Baraldi E. Early-life origins of chronic respiratory diseases: understanding and promoting healthy ageing. Eur Respir J. 2014;44(6):1682-96.
221. Fick A. Ueber Diffusion. Annalen der Physik. 1855;170(1):59-86.
222. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A, et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur Respir J. 2017;49(1).
223. Roughton FJ, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol. 1957;11(2):290-302.
224. Krogh M. The diffusion of gases through the lungs of man. J Physiol. 1915;49(4):271-300.
225. Hughes JM, Pride NB. Examination of the carbon monoxide diffusing capacity (DL(CO)) in relation to its KCO and VA components. Am J Respir Crit Care Med. 2012;186(2):132-9.
226. Hegewald MJ. Diffusing capacity. Clin Rev Allergy Immunol. 2009;37(3):159-66.
227. Ahlfeld SK, Conway SJ. Assessment of inhibited alveolar-capillary membrane structural development and function in bronchopulmonary dysplasia. Birth Defects Res A Clin Mol Teratol. 2014;100(3):168-79.
228. Newth CJ, Cotton DJ, Nadel JA. Pulmonary diffusing capacity measured at multiple intervals during a single exhalation in man. J Appl Physiol Respir Environ Exerc Physiol. 1977;43(4):617-25.
229. Wilson AF, Hearne J, Brenner M, Alfonso R. Measurement of transfer factor during constant exhalation. Thorax. 1994;49(11):1121-6.
230. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J. 2005;26(4):720-35.
231. Kanner RE, Crapo RO. The Relationship between Alveolar Oxygen-Tension and the Single-Breath Carbon-Monoxide Diffusing-Capacity. American Review of Respiratory Disease. 1986;133(4):676-8.
232. Smith TC, Rankin J. Pulmonary diffusing capacity and the capillary bed during Valsalva and Muller maneuvers. J Appl Physiol. 1969;27(6):826-33.
233. Frey TM, Crapo RO, Jensen RL, Elliott CG. Diurnal variation of the diffusing capacity of the lung: is it real? Am Rev Respir Dis. 1987;136(6):1381-4.

234. Sansores RH, Abboud RT, Kennell C, Haynes N. The effect of menstruation on the pulmonary carbon monoxide diffusing capacity. Am J Respir Crit Care Med. 1995;152(1):381-4.
235. Fitting JW. Transfer factor for carbon monoxide: a glance behind the scene. Swiss Med Wkly. 2004;134(29-30):413-8.
236. Ring AM, Carlens J, Bush A, Castillo-Corullon S, Fasola S, Gaboli MP, et al. Pulmonary function testing in children's interstitial lung disease. Eur Respir Rev. 2020;29(157).
237. Hughes JM. The single breath transfer factor (Tl,co) and the transfer coefficient (Kco): a window onto the pulmonary microcirculation. Clin Physiol Funct Imaging. 2003;23(2):63-71.
238. Nasr SZ, Amato P, Wilmott RW. Predicted values for lung diffusing capacity in healthy children. Pediatr Pulmonol. 1991;10(4):267-72.
239. Rosenthal M, Cramer D, Bain SH, Denison D, Bush A, Warner JO. Lung function in white children aged 4 to 19 years: II--Single breath analysis and plethysmography. Thorax. 1993;48(8):803-8.
240. Stam H, Beek AV, Grunberg K, de Ridder MA, de Jongste JC, Versprille A. A rebreathing method to determine carbon monoxide diffusing capacity in children: reference values for 6- to 18-year-olds [corrected] and validation in adult volunteers. Pediatr Pulmonol. 1998;25(3):205-12.
241. Koopman M, Zanen P, Kruitwagen CL, van der Ent CK, Arets HG. Reference values for paediatric pulmonary function testing: The Utrecht dataset. Respir Med. 2011;105(1):15-23.
242. Kim Y-J, Hall GL, Christoph K, Tabbey R, Yu Z, Tepper RS, et al. Pulmonary diffusing capacity in healthy caucasian children. Pediatric Pulmonology. 2012;47(5):469-75.
243. Thomas A, Hanel B, Marott JL, Buchvald F, Mortensen J, Nielsen KG. The Single-Breath Diffusing Capacity of CO and NO in Healthy Children of European Descent. PLOS ONE. 2014;9(12):e113177.
244. SHERRILL DL, ENRIGHT PL, KALTENBORN WT, LEBOWITZ MD. Predictors of Longitudinal Change in Diffusing Capacity over 8 Years. American Journal of Respiratory and Critical Care Medicine. 1999;160(6):1883-7.
245. Viegi G, Sherrill DL, Carrozzi L, Di Pede F, Baldacci S, Pistelli F, et al. An 8-year follow-up of carbon monoxide diffusing capacity in a general population sample of northern italy. Chest. 2001;120(1):74-80.
246. Storebo ML, Eagan TM, Eide GE, Gulsvik A, Thorsen E, Bakke PS. Change in pulmonary diffusion capacity in a general population sample over 9 years. Eur Clin Respir J. 2016;3:31265.
247. Stanojevic S, Graham BL, Cooper BG, Thompson BR, Carter KW, Francis RW, et al. Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians. Eur Respir J. 2017;50(3).
248. "Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians." Sanja Stanojevic, Brian L. Graham, Brendan G. Cooper, Bruce R. Thompson, Kim W. Carter, Richard

W. Francis and Graham L. Hall on behalf of the Global Lung Function Initiative T LCO working group. Eur Respir J 2017; 50: 1700010. Eur Respir J. 2020;56(4).
249. Punjabi NM, Shade D, Patel AM, Wise RA. Measurement variability in single-breath diffusing capacity of the lung. Chest. 2003;123(4):1082-9.
250. Welle I, Eide GE, Bakke P, Gulsvik A. Applicability of the single-breath carbon monoxide diffusing capacity in a Norwegian Community Study. Am J Respir Crit Care Med. 1998;158(6):1745-50.
251. Fitzgerald NM, Kennedy B, Fitzgerald DA, Selvadurai H. Diffusion capacity of carbon monoxide (DLCO) pre- and post-exercise in children in health and disease. Pediatr Pulmonol. 2014;49(8):782-9.
252. Moster D, Lie RT, Markestad T. Long-term medical and social consequences of preterm birth. N Engl J Med. 2008;359(3):262-73.
253. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet. 2008;371(9608):261-9.
254. Leversen KT, Sommerfelt K, Ronnestad A, Kaaresen PI, Farstad T, Skranes J, et al. Prediction of neurodevelopmental and sensory outcome at 5 years in Norwegian children born extremely preterm. Pediatrics. 2011;127(3):e630-8.
255. Thibeault DW, Mabry SM, Norberg M, Truog WE, Ekekezie, II. Lung microvascular adaptation in infants with chronic lung disease. Biol Neonate. 2004;85(4):273-82.
256. Doyle LW, Andersson S, Bush A, Cheong JLY, Clemm H, Evensen KAI, et al. Expiratory airflow in late adolescence and early adulthood in individuals born very preterm or with very low birthweight compared with controls born at term or with normal birthweight: a meta-analysis of individual participant data. Lancet Respir Med. 2019;7(8):677-86.
257. Balinotti JE, Chakr VC, Tiller C, Kimmel R, Coates C, Kisling J, et al. Growth of lung parenchyma in infants and toddlers with chronic lung disease of infancy. Am J Respir Crit Care Med. 2010;181(10):1093-7.
258. Chang DV, Assaf SJ, Tiller CJ, Kisling JA, Tepper RS. Membrane and Capillary Components of Lung Diffusion in Infants with Bronchopulmonary Dysplasia. Am J Respir Crit Care Med. 2016;193(7):767-71.
259. Castillo A, Llapur CJ, Martinez T, Kisling J, Williams-Nkomo T, Coates C, et al. Measurement of single breath-hold carbon monoxide diffusing capacity in healthy infants and toddlers. Pediatr Pulmonol. 2006;41(6):544-50.
260. Cazzato S, Ridolfi L, Bernardi F, Faldella G, Bertelli L. Lung function outcome at school age in very low birth weight children. Pediatr Pulmonol. 2013;48(8):830-7.
261. Hakulinen AL, Jarvenpaa AL, Turpeinen M, Sovijarvi A. Diffusing capacity of the lung in school-aged children born very preterm, with and without bronchopulmonary dysplasia. Pediatr Pulmonol. 1996;21(6):353-60.
262. Korhonen P, Laitinen J, Hyodynmaa E, Tammela O. Respiratory outcome in school-aged, very-low-birth-weight children in the surfactant era. Acta Paediatr. 2004;93(3):316-21.
263. Joshi S, Powell T, Watkins WJ, Drayton M, Williams EM, Kotecha S. Exercise-induced bronchoconstriction in school-aged children who had chronic lung disease in infancy. J Pediatr. 2013;162(4):813-8 e1.

264. Kaplan E, Bar-Yishay E, Prais D, Klinger G, Mei-Zahav M, Mussaffi H, et al. Encouraging pulmonary outcome for surviving, neurologically intact, extremely premature infants in the postsurfactant era. Chest. 2012;142(3):725-33.
265. Welsh L, Kirkby J, Lum S, Odendaal D, Marlow N, Derrick G, et al. The EPICure study: maximal exercise and physical activity in school children born extremely preterm. Thorax. 2010;65(2):165-72.
266. Lum S, Kirkby J, Welsh L, Marlow N, Hennessy E, Stocks J. Nature and severity of lung function abnormalities in extremely pre-term children at 11 years of age. Eur Respir J. 2011;37(5):1199-207.
267. Sorensen JK, Buchvald F, Berg AK, Robinson PD, Nielsen KG. Ventilation inhomogeneity and NO and CO diffusing capacity in ex-premature school children. Respir Med. 2018;140:94-100.
268. Arigliani M, Valentini E, Stocco C, De Pieri C, Castriotta L, Barbato V, et al. Regional ventilation inhomogeneity in survivors of extremely preterm birth. Pediatr Pulmonol. 2020;55(6):1366-74.
269. Wong PM, Lees AN, Louw J, Lee FY, French N, Gain K, et al. Emphysema in young adult survivors of moderate-to-severe bronchopulmonary dysplasia. Eur Respir J. 2008;32(2):321-8.
270. Vrijlandt EJ, Gerritsen J, Boezen HM, Grevink RG, Duiverman EJ. Lung function and exercise capacity in young adults born prematurely. Am J Respir Crit Care Med. 2006;173(8):890-6.
271. Um-Bergstrom P, Hallberg J, Pourbazargan M, Berggren-Brostrom E, Ferrara G, Eriksson MJ, et al. Pulmonary outcomes in adults with a history of Bronchopulmonary Dysplasia differ from patients with asthma. Respir Res. 2019;20(1):102.
272. Narang I, Bush A, Rosenthal M. Gas transfer and pulmonary blood flow at rest and during exercise in adults 21 years after preterm birth. Am J Respir Crit Care Med. 2009;180(4):339-45.
273. Drummond D, Hadchouel A, Le Bourgeois M, Roze JC, Marret S, Rousseau J, et al. Decreased pulmonary capillary volume in adolescents born very preterm. Acta Paediatr. 2020;109(3):621-2.
274. Low AT, Medford AR, Millar AB, Tulloh RM. Lung function in pulmonary hypertension. Respir Med. 2015;109(10):1244-9.
275. Balasubramanian A, Kolb TM, Damico RL, Hassoun PM, McCormack MC, Mathai SC. Diffusing Capacity Is an Independent Predictor of Outcomes in Pulmonary Hypertension Associated With COPD. Chest. 2020;158(2):722-34.
276. Balasubramanian A, MacIntyre NR, Henderson RJ, Jensen RL, Kinney G, Stringer WW, et al. Diffusing Capacity of Carbon Monoxide in Assessment of COPD. Chest. 2019;156(6):1111-9.
277. Hegewald MJ, Jensen RL, Teeter JG, Wise RA, Riese RJ, England RD, et al. Long-term intersession variability for single-breath diffusing capacity. Respiration. 2012;84(5):377-84.
278. Caruana EJ, Roman M, Hernandez-Sanchez J, Solli P. Longitudinal studies. J Thorac Dis. 2015;7(11):E537-40.

279. Halvorsen T. Lung sequelae after premature birth: A population based, controlled, long-term cohort study (Doctoral thesis). Bergen, Norway: University of Bergen; 2006.
280. Yudkin PL, Aboualfa M, Eyre JA, Redman CW, Wilkinson AR. New birthweight and head circumference centiles for gestational ages 24 to 42 weeks. Early Hum Dev. 1987;15(1):45-52.
281. RCoOa G. Preterm Prelabour Rupture of Membranes. RCOG Green-top Guideline; 2006.
282. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med. 2019;200(8):e70-e88.
283. Welsh EJ, Bara A, Barley E, Cates CJ. Caffeine for asthma. Cochrane Database Syst Rev. 2010(1):CD001112.
284. Selvaag AM, Ruperto N, Asplin L, Rygg M, Landgraf JM, Forre O, et al. The Norwegian version of the Childhood Health Assessment Questionnaire (CHAQ) and the Child Health Questionnaire (CHQ). Clin Exp Rheumatol. 2001;19(4 Suppl 23):S116-20.
285. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet. 1998;351(9111):1225-32.
286. Cotes JE, Chinn DJ, Quanjer PH, Roca J, Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity). Eur Respir J. 1993;6 Suppl 16:41-52.
287. Jones RS, Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. Q J Exp Physiol Cogn Med Sci. 1961;46:131-43.
288. Standardization of Spirometry - 1994 Update. American Journal of Respiratory and Critical Care Medicine. 1995;152(3):1107-36.
289. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung-Volumes and Forced Ventilatory Flows - Report Working Party Standardization of Lung-Function Tests European-Community for Steel and Coal - Official Statement of the European Respiratory Society. European Respiratory Journal. 1993;6:5-40.
290. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
291. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, et al. Reference ranges for spirometry across all ages: a new approach. Am J Respir Crit Care Med. 2008;177(3):253-60.
292. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. Eur Respir J. 2005;26(3):511-22.
293. Stocks J, Quanjer PH. Reference values for residual volume, functional residual capacity and total lung capacity. ATS Workshop on Lung Volume Measurements. Official Statement of The European Respiratory Society. Eur Respir J. 1995;8(3):492-506.

294. Halvorsen T, Skadberg BT, Eide GE, Roksund OD, Bakke P, Thorsen E. Assessment of lung volumes in children and adolescents: comparison of two plethysmographic techniques. Clin Physiol Funct Imaging. 2005;25(1):62-8.
295. Cumming GR, Everatt D, Hastman L. Bruce treadmill test in children: normal values in a clinic population. Am J Cardiol. 1978;41(1):69-75.
296. American Thoracic S, American College of Chest P. ATS/ACCP Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med. 2003;167(2):211-77.
297. Armstrong N, Welsman J, Winsley R. Is peak VO2 a maximal index of children's aerobic fitness? International Journal of Sports Medicine. 1996;17(5):356-9.
298. Jones NL, Makrides L, Hitchcock C, Chypchar T, McCartney N. Normal standards for an incremental progressive cycle ergometer test. Am Rev Respir Dis. 1985;131(5):700-8.
299. Clemm HH, Vollsaeter M, Roksund OD, Markestad T, Halvorsen T. Adolescents who were born extremely preterm demonstrate modest decreases in exercise capacity. Acta Paediatr. 2015;104(11):1174-81.
300. Clemm HH, Vollsaeter M, Roksund OD, Eide GE, Markestad T, Halvorsen T. Exercise capacity after extremely preterm birth. Development from adolescence to adulthood. Ann Am Thorac Soc. 2014;11(4):537-45.
301. Clemm H, Roksund O, Thorsen E, Eide GE, Markestad T, Halvorsen T. Aerobic capacity and exercise performance in young people born extremely preterm. Pediatrics. 2012;129(1):e97-e105.
302. Pettersen MD, Du W, Skeens ME, Humes RA. Regression equations for calculation of z scores of cardiac structures in a large cohort of healthy infants, children, and adolescents: an echocardiographic study. J Am Soc Echocardiogr. 2008;21(8):922-34.
303. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020.
304. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. 2015. 2015;67(1):48.
305. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10.
306. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res. 1999;8(2):135-60.
307. Mallinckrodt CH, Clark WS, David SR. ACCOUNTING FOR DROPOUT BIAS USING MIXED-EFFECTS MODELS. Journal of Biopharmaceutical Statistics. 2001;11(1-2):9-21.
308. Vollsæter M. Long-term Respiratory Outcomes of Extreme Preterm Birth. A regional cohort study. Bergen, Norway: University of Bergen; 2016.
309. Lee JY, Ahn TG, Jun JK. Short-Term and Long-Term Postnatal Outcomes of Expectant Management After Previable Preterm Premature Rupture of Membranes With and Without Persistent Oligohydramnios. Obstetrics & Gynecology. 2015;126(5):947-53.
310. Bentsen MH, Markestad T, Oymar K, Halvorsen T. Lung function at term in extremely preterm-born infants: a regional prospective cohort study. BMJ Open. 2017;7(10):e016868.

311. MacLean JE, DeHaan K, Fuhr D, Hariharan S, Kamstra B, Hendson L, et al. Altered breathing mechanics and ventilatory response during exercise in children born extremely preterm. Thorax. 2016;71(11):1012-9.
312. Storebø ML, Universitetet i Bergen Klinisk i, Universitetet i B. Diffusing capacity of the lung for carbon monoxide : variability and longitudinal changes over nine years. Bergen, Norway: University of Bergen; 2019.
313. Heerink WJ, de Bock GH, de Jonge GJ, Groen HJM, Vliegenthart R, Oudkerk M. Complication rates of CT-guided transthoracic lung biopsy: meta-analysis. European Radiology. 2017;27(1):138-48.
314. Sobrino JA, Le N, Sujka JA, Benedict LA, Rentea RM, Alemayehu H, et al. Therapeutic Direction Versus Adverse Outcomes in Children Undergoing Lung Biopsy. Journal of Surgical Research. 2019;236:106-9.
315. Satrell E, Roksund O, Thorsen E, Halvorsen T. Pulmonary gas transfer in children and adolescents born extremely preterm. Eur Respir J. 2013;42(6):1536-44.
316. Cotes JE, Dabbs JM, Elwood PC, Hall AM, McDonald A, Saunders MJ. Iron-deficiency anaemia: its effect on transfer factor for the lung (diffusiong capacity) and ventilation and cardiac frequency during sub-maximal exercise. Clin Sci. 1972;42(3):325-35.
317. Bruserud IS, Roelants M, Oehme NHB, Madsen A, Eide GE, Bjerknes R, et al. References for Ultrasound Staging of Breast Maturation, Tanner Breast Staging, Pubic Hair, and Menarche in Norwegian Girls. The Journal of Clinical Endocrinology & Metabolism. 2020;105(5):1599-607.
318. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005;26(5):948-68.
319. Stanojevic S, Quanjer P, Miller MR, Stocks J. The Global Lung Function Initiative: dispelling some myths of lung function test interpretation. Breathe. 2013;9(6):462-74.
320. Rosenthal M, Cramer D, Bain SH, Denison D, Bush A, Warner JO. Lung function in white children aged 4 to 19 years: II--Single breath analysis and plethysmography. Thorax. 1993;48(8):803-8.
321. Cotes JE, Dabbs JM, Hall AM, Axford AT, Laurence KM. Lung volumes, ventilatory capacity, and transfer factor in healthy British boy and girl twins. Thorax. 1973;28(6):709-15.
322. Nasr SZ, Amato P, Wilmott RW. Predicted values for lung diffusing capacity in healthy children. Pediatric Pulmonology. 1991;10(4):267-72.
323. Stam H, v. d. Beek A, Grünberg K, de Ridder MAJ, de Jongste JC, Versprille A. A rebreathing method to determine carbon monoxide diffusing capacity in children: Reference values for six 18-year-olds and validation in adult volunteers. Pediatric Pulmonology. 1998;25(3):205-12.
324. Koopman M, Zanen P, Kruitwagen CLJJ, van der Ent CK, Arets HGM. Reference values for paediatric pulmonary function testing: The Utrecht dataset. Respiratory Medicine. 2011;105(1):15-23.
325. Walsh MC, Wilson-Costello D, Zadell A, Newman N, Fanaroff A. Safety, reliability, and validity of a physiologic definition of bronchopulmonary dysplasia. J Perinatol. 2003;23(6):451-6.

326. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. Am Rev Respir Dis. 1991;144(5):1202-18.
327. Hankinson JL, Stocks J, Peslin R. Reproducibility of lung volume measurements. Eur Respir J. 1998;11(3):787-90.
328. Drummond MB, Schwartz PF, Duggan WT, Teeter JG, Riese RJ, Ahrens RC, et al. Intersession variability in single-breath diffusing capacity in diabetics without overt lung disease. Am J Respir Crit Care Med. 2008;178(3):225-32.
329. Hathaway EH, Tashkin DP, Simmons MS. Intraindividual variability in serial measurements of DLCO and alveolar volume over one year in eight healthy subjects using three independent measuring systems. Am Rev Respir Dis. 1989;140(6):1818-22.
330. Punjabi NM, Shade D, Patel AM, Wise RA. Measurement Variability in Single-Breath Diffusing Capacity of the Lung. Chest. 2003;123(4):1082-9.
331. Kiss D, Popp W, Wagner C, Havelec L, Sertl K. Comparison of the single breath with the intrabreath method for the measurement of the carbon monoxide transfer factor in subjects with and without airways obstruction. Thorax. 1995;50(8):902-5.
332. Quantz M, Wilson S, Smith C, Stitt L, Novick R, Ahmad D. Advantages of the Intrabreath Technique as a Measure of Lung Function Before and After Heart Transplantation*. Chest. 2003;124(5):1658-62.
333. Satrell E, Clemm H, Roksund O, Hufthammer KO, Thorsen E, Halvorsen T, et al. Development of lung diffusion to adulthood following extremely preterm birth. Eur Respir J. 2021.
334. Kilbride HW, Gelatt MC, Sabath RJ. Pulmonary function and exercise capacity for ELBW survivors in preadolescence: effect of neonatal chronic lung disease. The Journal of pediatrics. 2003;143(4):488-93.
335. Burns YR, Danks M, O'Callaghan MJ, Gray PH, Cooper D, Poulsen L, et al. Motor coordination difficulties and physical fitness of extremely-low-birthweight children. Dev Med Child Neurol. 2009;51(2):136-42.
336. Vrijlandt EJ, Gerritsen J, Boezen HM, Grevink RG, Duiverman EJ. Lung function and exercise capacity in young adults born prematurely. AmJRespirCrit Care Med. 2006;173(8):890-6.
337. Association AM. Guides to the Evaluation of Permanent Impairment. Chicago: American Medical Association; 2008.
338. Moschino L, Stocchero M, Filippone M, Carraro S, Baraldi E. Longitudinal Assessment of Lung Function in Survivors of Bronchopulmonary Dysplasia from Birth to Adulthood. The Padova BPD Study. Am J Respir Crit Care Med. 2018;198(1):134-7.
339. Balasubramanian A, Kolb TM, Damico RL, Hassoun PM, McCormack MC, Mathai SC. Diffusing Capacity Is an Independent Predictor of Outcomes in Pulmonary Hypertension Associated With COPD. Chest. 2020.
340. Balasubramanian A, Putcha N, MacIntyre NR, Jensen RL, Kinney G, Stringer WW, et al. Diffusing Capacity and Mortality in Chronic Obstructive Pulmonary Disease. Ann Am Thorac Soc. 2022.
341. Neas LM, Schwartz J. Pulmonary function levels as predictors of mortality in a national sample of US adults. Am J Epidemiol. 1998;147(11):1011-8.

342. Kitterman JA. Physiological factors in fetal lung growth. Can J Physiol Pharmacol. 1988;66(8):1122-8.
343. Moessinger AC, Harding R, Adamson TM, Singh M, Kiu GT. Role of lung fluid volume in growth and maturation of the fetal sheep lung. J Clin Invest. 1990;86(4):1270-7.
344. Mourani PM, Abman SH. Pulmonary Hypertension and Vascular Abnormalities in Bronchopulmonary Dysplasia. Clin Perinatol. 2015;42(4):839-55.
345. Austin ED, Kawut SM, Gladwin MT, Abman SH. Pulmonary Hypertension: NHLBI Workshop on the Primary Prevention of Chronic Lung Diseases. Annals of the American Thoracic Society. 2014;11(Supplement 3):S178-S85.

Appendix

Appendix I

1. OM BARNETS HELSE

1.1 Er barnet funksjonshemmet på noen av disse måtene:
(Sett ett kryss på hver linje for det som passer best)

Er bevegelseshemmet:
☐ Nei ☐ Litt ☐ Middels ☐ Mye

Har nedsatt syn:
☐ Nei ☐ Litt ☐ Middels ☐ Mye

Har nedsatt hørsel:
☐ Nei ☐ Litt ☐ Middels ☐ Mye

Hemmet på grunn av kroppslig sykdom:
☐ Nei ☐ Litt ☐ Middels ☐ Mye

Hemmet på grunn av psykiske plager:
☐ Nei ☐ Litt ☐ Middels ☐ Mye

1.2 Har barnet noen gang hatt tung pust eller piping/surkling/tetthet i brystet ?
☐ ja
☐ nei
Hvis du har svart nei, gå til spørsmål 1.7

1.3 Har barnet hatt tung pust eller piping/surkling/tetthet i brystet i løpet av de siste 12 måneder ?
☐ ja
☐ nei
Hvis du har svart nei, gå til spørsmål 1.7

1.4 Hvor mange anfall av tung pust eller piping/surkling/tetthet i brystet har barnet hatt i løpet av de siste 12 måneder ?
☐ ingen
☐ 1 til 3
☐ 4 til 12
☐ mer enn 12

1.5 Hvor ofte har barnets søvn i gjennomsnitt blitt forstyrret på grunn av tung pust eller piping/surkling/tetthet i brystet de siste 12 måneder ?
☐ aldri våknet
☐ mindre enn 1 natt pr. uke
☐ 1 eller flere netter pr. uke

1.6 Har piping/surkling/tetthet i brystet eller tung pust vært så alvorlig de siste 12 måneder at barnet har hatt problemer med å snakke slik at han/hun bare kunne si ett eller to ord mellom hvert pust ?

☐ ja
☐ nei

1.7 Har barnet noen gang hatt astma ?

☐ ja
☐ nei

1.8 Har barnet i løpet av de siste 12 måneder hatt tung pust eller piping/surkling/tetthet i brystet under eller etter fysisk trening, aktiv lek eller mosjonering ?

☐ ja
☐ nei

1.9 Har barnet i løpet av de siste 12 måneder hatt tørr hoste om natten, utenom hoste i forbindelse med en forkjølelse eller andre luftveisinfeksjoner ?

☐ ja
☐ nei

Nå følger en rekke litt mer detaljerte spørsmål om luftveier. Noen ligner litt på de du allerede har besvart, men ingen er helt like.

1.10 Har barnet noen gang av lege har fått diagnosen astma:

☐ ja ☐ nei

Hvis ja, svar på følgende:

Hva var barnets alder da han/hun fikk diagnosen: _____ år _____ måneder

Hvis han/hun er friskt nå, ved hvilken alder forsvant astmaen: _____ år _____ måneder

1.11 Har barnets søvn noen gang blitt forstyrret på grunn av tung pust eller piping/surkling/tetthet i brystet:

☐ nei
☐ ja, noen ganger (færre enn 10)
☐ ja, mange ganger (10 eller flere ganger)

1.12 Har barnet hatt nattlig hoste de siste 12 måneder (uansett årsak) :

☐ nei
☐ ja, men ikke så ofte som hver måned
☐ ja, hver måned, men ikke så ofte som hver uke
☐ ja, hver uke

1.13 Har barnet hatt tett nese eller rennende nese uten være forkjølet de siste 12 måneder:

☐ nei
☐ ja, men ikke så ofte som hver måned
☐ ja, hver måned, men ikke så ofte som hver uke
☐ ja, hver uke

1.14 Har barnet hatt noen av følgende sykdommer i løpet av de siste 12 måneder og i tilfelle hvor mange ganger:

Forkjølelse	☐ nei	☐ ja, antall ganger	_____
Halsbetennelse	☐ nei	☐ ja, antall ganger	_____
Øreverk	☐ nei	☐ ja, antall ganger	_____
Bihulebetennelse	☐ nei	☐ ja, antall ganger	_____
Bronkitt	☐ nei	☐ ja, antall ganger	_____
Lungebetennelse	☐ nei	☐ ja, antall ganger	_____

1.15 Oppgi antall episoder med tung pust eller piping/surkling/tetthet i brystet barnet har hatt ved de alderstrinn som er angitt under:

Oppgi antallet som: 0 eller 1-3 eller 4-10 eller flere enn 10

	Barnets alderstrinn:				
	Under 1 år	1-2 år	3-5 år	6-12 år	Eldre enn 12 år
Antall episoder	|_____|	|_____|	|_____|	|_____|	|_____|

1.16 Har barnet noen gang hatt atopisk eksem (kløende barne-eksem) ☐ nei ☐ ja

1.17 Har barnet noen gang hatt høysnue (allergi i øyne eller nese) : ☐ nei ☐ ja

1.18 Har barnet noen gang:

Fjernet mandlene	☐ nei	☐ ja
Fjernet de falske mandlene / "polyppene"	☐ nei	☐ ja
Stukket hull på trommehinnen	☐ nei	☐ ja
Lagt inn dren i trommehinnen	☐ nei	☐ ja

1.19 Har barnet noen gang hatt øreverk:

☐ Nei, aldri
☐ Ja, 1 til 3 ganger
☐ Ja, 4 til 10 ganger
☐ Ja, flere enn 10 ganger

1.20 Har barnet <u>noen gang</u> hatt falsk krupp

☐ Nei, aldri
☐ Ja, 1 til 3 ganger
☐ Ja, 4 til 10 ganger
☐ Ja, flere enn 10 ganger

Hvis ja, angi barnets alder ved den **siste** episoden:

_____ år _____ måneder

1.21 Har barnet <u>noen gang</u> hatt lungebetennelse:

☐ Nei, aldri
☐ Ja, 1 til 3 ganger
☐ Ja, 4 til 10 ganger
☐ Ja, flere enn 10 ganger

1.22 Har barnet <u>noen gang</u> vært innlagt på sykehus på grunn av sykdommer i luftveiene:

☐ Nei, aldri
☐ Ja, 1 til 3 ganger
☐ Ja, 4 til 10 ganger
☐ Ja, flere enn 10 ganger

1.23 Hvis barnet noen gang har vært innlagt på sykehus - <u>uansett hvorfor</u>, angi <u>årsak</u> og <u>antall</u> ganger:

	Barnets alderstrinn: Under 1 år	1-2 år	3-5 år	6 -12 år	Eldre enn 12 år
Årsaker:					
• Astma, bronkitt eller bronkiolitt	|_____|	|_____|	|_____|	|_____|	|_____|
• Lunge-betennelse	|_____|	|_____|	|_____|	|_____|	|_____|
• Falsk krupp	|_____|	|_____|	|_____|	|_____|	|_____|
• Febersyk	|_____|	|_____|	|_____|	|_____|	|_____|
• Andre årsaker	|_____|	|_____|	|_____|	|_____|	|_____|

Angi i så fall hvilke(n): _____

1.24 Har barnet normalt syn: ☐ nei ☐ ja

1.25 Trenger barnet briller nå: ☐ nei ☐ ja

1.26 Har barnet tidligere hatt behov for briller: ☐ nei ☐ ja

1.27 Skjeler barnet: ☐ nei ☐ ja

1.28 Har barnet normal hørsel: ☐ nei ☐ ja

1.29 Bruker barnet noen form for høreapparat: ☐ nei ☐ ja

1.30 Har barnet tidligere hatt behov for høreapparat: ☐ nei ☐ ja

1.31 Har barnet noen medfødte misdannelser: ☐ nei ☐ ja

Hvis ja, angi hva_____

1.32 Er barnet vaksinert etter vanlig rutiner: ☐ nei ☐ ja

2. OM BARNETS FYSISKE AKTIVITET

2.1 Utenom skoletid, hvor ofte driver barnet idrett eller mosjonerer så mye at han/hun blir andpusten og/eller svett:

☐ hver dag
☐ 4-6 ganger i uken
☐ 2-3 ganger i uken
☐ en gang i uken
☐ 1-3 ganger i måneden
☐ mindre enn en gang i måneden
☐ aldri

2.2 Utenom skoletid, hvor mange timer i uken driver barnet idrett eller mosjonerer eller anstrenger seg så mye at han/hun blir andpusten og/eller svett:

☐ ingen
☐ omtrent 1/2 time
☐ omtrent 1 time
☐ omtrent 2-3 timer
☐ omtrent 4-6 timer
☐ 7 timer eller mer

2.3 Er barnet aktivt medlem av idrettslag: ☐ nei ☐ ja

2.4 Har barnet noen gang hatt tung pust eller piping/surkling/tetthet i brystet under eller etter fysisk trening, mosjonering eller aktiv lek:

☐ nei ☐ ja

2.5 Har hoste etter anstrengelse/trening noen gang vært et problem for barnet:

☐ nei, aldri
☐ ja, tidligere - for mer enn 12 måneder siden
☐ ja, i løpet av de siste 12 måneder

2.6 Har barnet noen gang hatt diagnosen anstrengelses-utløst astma:

☐ nei, aldri
☐ ja, tidligere - for mer enn 12 måneder siden
☐ ja, i løpet av de siste 12 måneder

2.7 Har barnet noen gang brukt astmamedisin før anstrengelse:

☐ nei, aldri
☐ ja, tidligere - for mer enn 12 måneder siden
☐ ja, i løpet av de siste 12 måneder

2.8 Hvor ofte har barnet avbrudd i fysisk aktivitet eller trening pga. sykdom:

☐ Hver uke
☐ Hver måned
☐ Hver annen måned
☐ Hvert halvår
☐ Sjeldnere enn hvert halvår
☐ Aldri

2.9 Opplever barnet pustebesvær i forbindelse med anstrengelse:

☐ nei ☐ ja
Dersom ja:

* Er pustebesværet verst under anstrengelse eller rett etter anstrengelse:
  ☐ under ☐ etter ☐ vet ikke

* Er pustebesværet mest utpreget på ut-pust eller inn-pust:
  ☐ ut-pust ☐ inn-pust ☐ vet ikke

* Er pustebesværet ledsaget av smerter i brystet:
  ☐ nei ☐ ja ☐ vet ikke

3. OM BRUK AV MEDISINER

3.1 Har barnet noen gang brukt medisiner mot:

Hoste: ☐ nei ☐ ja
Tung, tett, surklete eller pipete pust: ☐ nei ☐ ja

3.2 Har barnet noen gang brukt medisiner mot astma:

☐ Nei
☐ Ja, av og til
☐ Ja, regelmessig i mer enn 3 måneder

Hvis ja, har barnet brukt medisiner mot astma **de siste 12 månedene:** ☐ nei ☐ ja

3.3 Har barnet noen gang brukt noen av de medisinene som er listet opp under: ☐ nei ☐ ja

Hvis ja, kryss av ved hvilke alderstrinn
Du krysser av for alle alderstrinn som barnet har brukt de ulikemedisintypene

MEDISINTYPER:	**BARNETS ALDERS-TRINN:**			
Miksturer, dvs flytende til å drikke	Under 1 år	1-5 år	6-12 år	Eldre enn 12 år
Hostesaft	☐	☐	☐	☐
Efedrin, Bricanyl, Ventoline, Salbuvent	☐	☐	☐	☐
Astma-medisin til inhalasjon (til å puste inn)	Under 1 år	1-5 år	6-12 år	Eldre enn 12 år
Bricanyl, Ventoline, Salbuvent	☐	☐	☐	☐
Lomudal	☐	☐	☐	☐
Becotide, Pulmicort, Flutide	☐	☐	☐	☐
Serevent eller Oxis	☐	☐	☐	☐
Seretide	☐	☐	☐	☐
Astma-tabletter eller klyster	Under 1 år	1-5 år	6-12 år	Eldre enn 12 år
Teovent klyster eller theodur	☐	☐	☐	☐
Singulair tabletter	☐	☐	☐	☐
Allergi-medisiner F. eks. Zyrtec, Teldanex, Clarityn, Polaramin, Phenamin, Vallergan, Phenergan	Under 1 år ☐	1-5 år ☐	6-12 år ☐	Eldre enn 12 år ☐
Andre medisiner mot astma og/eller pustebesvær	☐	☐	☐	☐

Angi hva _____

4. OM BARNETS TILSYN OG SKOLEGANG

4.1 Hva slags tilsyn hadde barnet før skolealder. Dersom delte ordninger, kryss for alle:

	Barnets alderstrinn:		
	Under 1 år	1-2 år	3-5 (6) år
Hjemme	☐	☐	☐
Praktikant/dagmamma	☐	☐	☐
Barnehage/park	☐	☐	☐
Antall barn som var sammen	____	_____	_____

4.2 Hvis barnet gikk i barnepark eller barnehage, fikk det plassen på grunn av sykdom hos barnet:

☐ Nei
☐ Ja, angi årsak _____

4.3 Hvis barnet gikk i barnepark eller barnehage, mottok barnet noen form for hjelp for barn med spesielle behov:

☐ Nei
☐ Ja, angi hva _____

4.4 Hvis barnet ikke fikk slik ekstra hjelp, mener du/dere at barnet burde hatt det: ☐ nei ☐ ja

4.5 Har barnet gått i vanlig skole: ☐ nei ☐ ja

Hvis nei, angi type skole: _____

4.6 Har barnet fått støtteundervisning på skolen: ☐ nei ☐ ja

Hvis nei, mener du/dere at barnet burde hatt slik støtteundervisning: ☐ nei ☐ ja

4.7 Når det gjelder skolearbeidet, hvordan har barnet klart seg:

☐ omtrent som andre barn i klassen
☐ bedre enn andre barn i klassen
☐ ikke så bra som andre barn i klassen

4.8 Har barnet hatt lese – og skrivevansker ☐ nei ☐ ja

4.9 Når det gjelder gymnastikktimene, hvordan har barnet klart seg:

☐ omtrent som andre barn i klassen
☐ bedre enn andre barn i klassen
☐ ikke så bra som andre barn i klassen

4.10 Prøv å angi barnets fravær i grunnskolen på grunn av sykdom:

☐ Ukentlig
☐ Månedlig
☐ Hver annen måned
☐ Hvert halvår
☐ Sjeldnere enn hvert halvår

4.11 Har barnet vært i kontakt med PPT (Pedagogisk Psykologisk Tjeneste):

☐ nei ☐ ja

4.12 Hva har barnet gjort etter grunnskolen, dvs 9 / 10 klasse:
(besvares bare av de som er ferdig med grunnskolen)

☐ Gått videregående skole, allmennfaglig
☐ Gått videregående skole, yrkesfaglig
☐ Begynt på videregående skole, men sluttet
☐ Hovedsakelig vært i arbeid
☐ Annet, angi hva:_____
_____

5. OM FAMILIEN

5.1 Hvem har barnet bodd sammen med gjennom oppveksten:

☐ Mor og far
☐ Mest hos mor
☐ Mest hos far
☐ Andre omsorgsgivere, hvem _____

5.2 Hvor mange søsken har barnet (hel og halvsøsken): _____

Hvilken plass har barnet i søsken-rekkefølgen:
☐ eldst ☐ yngst ☐ midt i mellom

Hvor mange av barnets søsken er født
tidligere enn 34 svangerskapsuke: _____

5.3 Har barnets mor, far eller søsken noen gang hatt astma:
☐ nei ☐ ja

Dersom ja, hvem:
☐ Mor
☐ Far
☐ Søsken, angi hvor mange med astma: _____

5.4 Har barnets mor, far eller søsken noen gang hatt atopisk eksem (kløende barne-eksem) ☐ nei ☐ ja

Dersom ja, hvem:
☐ Mor
☐ Far
☐ Søsken, angi hvor mange med atopisk eksem: _____

5.5 Har barnets mor, far eller søsken noen gang hatt høysnue (allergi i øyne/nese) ☐ nei ☐ ja

Dersom ja, hvem:
☐ Mor
☐ Far
☐ Søsken, angi hvor mange med høysnue: _____

5.6 Hvilken utdannelse har mor:

☐ Grunnskole
☐ Videregående skole
☐ Høyskole / universitet 4 år eller mindre
☐ Høyskole / universitet 4 år eller mer
☐ Annet (spesifiser)_____
_____

5.7 Hvilken utdannelse har far:

☐ Grunnskole
☐ Videregående skole
☐ Høyskole / universitet 4 år eller mindre
☐ Høyskole / universitet 4 år eller mer
☐ Annet (spesifiser))_____
_____

5.8 Er noen av omsorgspersonene i husholdningen (mor, far eller samboer) mottaker av:

☐ Uførepensjon
☐ Arbeidsledighetstrygd
☐ Overgangsstønad til enslige forsørger
☐ Under attføring

6. OM HJEMMET

6.1 Hvor mange personer bodde barnet sammen med gjennom oppveksten:

	Barnets alderstrinn			
	Under 1 år	1-5 år	6-12 år	mer enn 12 år
Antall personer i husstanden	|_____|	|_____|	|____|	|_____|

6.2 Har familien hatt hund eller katt eller andre husdyr:

	Barnets alder:			
	Under 1 år	1-5 år	6-12 år	mer enn 12 år
Nei	☐	☐	☐	☐
Hund	☐	☐	☐	☐
Katt	☐	☐	☐	☐
Andre dyr	☐	☐	☐	☐

7. OM RØYKING

7.1 **Røykte mor under svangerskapet**
☐ Nei
☐ Ja, angi antall sigaretter per dag: _____

7.2 **Hvis mor sluttet å røyke under svangerskapet,**
i hvilken graviditets-måned sluttet hun: _____ måned

7.3 **Røykte far under svangerskapet:**
☐ Nei
☐ Ja, angi antall sigaretter per dag: _____

7.4 **Har det noen gang blitt røykt daglig i barnets hjem eller der barnet har oppholdt seg til daglig:** ☐ nei ☐ ja

Dersom ja, av hvem og når:	Mor	Far	Andre
I løpet av de siste 12 måneder:	☐	☐	☐
Ved barnets fødsel:	☐	☐	☐
Barnets første leveår:	☐	☐	☐

8. OM AMMING

8.1 **Har barnet noen gang fått morsmelk:** ☐ nei ☐ ja
Hvis ja, i hvor mange måneder ble barnet ammet:
☐ Mindre enn 3 måneder
☐ 3 til 6 måneder
☐ 7 til 12 måneder
☐ Mer enn ett år

Hvis ja, hvor lenge fikk barnet <u>bare</u> morsmelk, <u>uten tillegg av annen mat/drikke</u>
☐ Mindre enn 2 måneder
☐ 2 til 4 måneder
☐ 5 til 6 måneder
☐ Mer enn 6 måneder

9. SPØRSMÅL TIL FORELDRENE OM HVORDAN SYKDOM HOS BARNET HAR PÅVIRKET FAMILIEN

9.1 **Har sykdom hos barnet ditt noen gang påvirket din egen utdannelse og /eller arbeidssituasjon (sett ett kryss):**
☐ ikke i det hele tatt ☐ noe ☐ svært mye
☐ svært lite ☐ ganske mye

Hvor gammelt var barnet da problemene eventuelt var størst: _____ år

9.2 **Har sykdom hos barnet ditt noen gang påvirket din egen fritid (sett ett kryss):**
☐ ikke i det hele tatt ☐ noe ☐ svært mye

☐ svært lite ☐ ganske mye

Hvor gammelt var barnet da problemene eventuelt var størst: _____ år

9.3 **Har familien mottatt hjelpestønad fra Folketrygden på grunn av barnet**: ☐ nei ☐ ja
Hvor gammelt var barnet da familien fikk hjelpestønad: _____ år

9.4 **Har familien mottatt grunnstønad fra Folketrygden på grunn av barnet:** ☐ nei ☐ ja
Hvor gammelt var barnet da familien fikk grunnstønad: _____ år

9.5 **Har familien mottatt andre ytelser fra kommunen eller det offentlige på grunn av barnet:** ☐ nei ☐ ja
Hvis ja, angi hva slags ytelser _____
_____
Hvor gammelt var barnet da familien fikk slik støtte: _____ år

9.6 **Mener du/dere at barnet gjennom oppveksten burde hatt mer hjelp, eller blitt bedre fulgt opp fra helsevesenet, trygdesystemet, kommunen eller andre offentlige støtte/tilsynsordninger**: ☐ nei ☐ ja

Hvis ja, beskriv: _____
_____

10. OM DEN SOM FYLLER UT SKJEMA

10.1 **Kjønn:** ☐ **Mann** ☐ **Kvinne** **Alder:** _____ **år**

10.2 **Har du i den senere tid vært gjennom viktige eller dramatiske hendelser som for eksempel fått barn, opplevd alvorlig sykdom, dødsfall eller fødsler i den nære familie eller blant nære venner, giftet deg eller endret sosial status på annen måte eller lignende:** ☐ **nei** ☐ **ja**

10.3 **Har du noen gang opplevd å miste et barn:** ☐ nei ☐ ja

Hvis ja, når: _____ Hvor gammelt var barnet: _____ år
Var barnet for tidlig født: ☐ nei ☐ ja

10.4 Hvem bor du sammen med? (Kryss av for den/de du bor sammen med.)

- ☐ Bor alene
- ☐ Ektefelle eller samboer
- ☐ Foreldre eller svigerforeldre
- ☐ Barnet som er født for tidlig
- ☐ Øvrige barn
- ☐ Andre voksne personer

Takk for at du fylte ut skjema !

Dersom du har kommentarer som ikke passer inn i de ferdige svaralternativene, er du velkommen til å bruke baksiden på arkene.

Rev. 11.12.17

PREMATURSTUDIEN VED BARNEKLINIKKEN I BERGEN

UNDERSØKELSE AV LUNGEFUNKSJON, ARBEIDSKAPASITET OG LIVSKVALITET HOS BARN SOM ER FØDT FOR TIDLIG

Spørreskjema for voksne

Løpenummer (fylles ut på Barneklinikken): ☐☐ - ☐☐☐

Kjære deltaker

Takk for at du vil være med i denne undersøkelsen. I tillegg til lungeundersøkelsen, ønsker vi at du skal fylle ut noen spørreskjemaer som kan fortelle oss hvordan du har det. Det finnes ikke riktige eller gale svar. Det er viktig at du finner det alternativet som passer best for deg.

Du synes kanskje det er mange spørsmål, og at noen kan være vanskelig å svare på. Gjør så godt du kan og ta den tiden du trenger! Prøv å svar i den rekkefølgen spørsmålene står. Les spørsmål og forklaringer nøye.

Selv om noen av spørsmålene kan se like ut, er det viktig at du svarer på alle. Når du er ferdig, ber vi deg se etter om du har svart på alle spørsmålene.

Du svarer ved å krysse av i rutene. Hvis det er lov å sette flere enn ett kryss, vil du se at vi har skrevet det i parentes etter spørsmålet.

Dine svar vil ikke bli vist til noen.

Takk for at du tar deg tid til å fylle ut spørreskjemaet !

Om deg selv

1. Kjønn
☐ Mann ☐ Kvinne

2. Alder
☐☐ år

3. Hvem bor du sammen med? *(sett ett eller flere kryss)*
☐ Ingen
☐ Ektefelle /samboer
☐ Foreldre
☐ Andre personer over 18 år
☐ Personer under 18 år
☐ Institusjon / bofellesskap med tilsyn
☐ Egne barn

Om utdanning og arbeid

4. Hvilken utdanning er den høyeste du har fullført? *(bare ett kryss)*
☐ Grunnskole 7-10 år
☐ Videregående skole, allmennfaglig
☐ Videregående skole, yrkesfaglig
☐ Høgskole/universitet, mindre enn 4 år
☐ Høgskole/universitet, 4 år eller mer

5. Hva slags arbeidssituasjon har du nå? *(sett ett eller flere kryss)*
☐ Lønnet arbeid
☐ Deltid
☐ Selvstendig næringsdrivende
☐ Heltids husarbeid
☐ Utdanning, militærtjeneste
☐ Arbeidsledig, permittert
☐ Uføretrygdet
☐ Annet, ***Spesifiser***_____

6. Mottar du noen av følgende *offentlige* ytelser?

•Sykepenger/sykelønn/rehabiliteringspenger	☐ Ja	☐ Nei
•Ytelser under yrkesrettet attføring	☐ Ja	☐ Nei
•Uførepensjon	☐ Ja	☐ Nei
•Sosialstøtte	☐ Ja	☐ Nei
•Arbeidsløshetstrygd	☐ Ja	☐ Nei
•Andre ytelser	☐ Ja	☐ Nei

7. Har du i løpet av de siste 12 månedene hatt sykefravær?

Med egenmelding	☐ Ja	☐ Nei
Med sykmelding fra lege	☐ Ja	☐ Nei

Hvis JA: 100% sykmeldt☐ delvis sykmeldt☐

8. *Hvis JA:* Hvor lenge til sammen? *(bare ett kryss)*
☐ 2 uker eller mindre
☐ 2-8 uker
☐ Mer enn 8 uker

9.

	Omtrent som de fleste andre	Bedre råd	Dårligere råd
Hvor god råd synes du at du/familien din har i forhold til de fleste andre?	☐	☐	☐

Om fritid/aktivitet

10. Hvor ofte driver du idrett eller mosjonerer så mye at du blir andpusten og/eller svett?
☐ Hver dag
☐ 4-6 ganger i uken
☐ 2-3 ganger i uken
☐ En gang i uken
☐ Mindre enn en gang i uken
☐ Aldri

11. Hvor mange timer i uken driver du idrett eller mosjonerer eller anstrenger deg så mye at du blir andpusten og/eller svett:
☐ Ingen
☐ Omtrent 1/2 time
☐ Omtrent 1 time
☐ Omtrent 2-3 timer
☐ Omtrent 4-6 timer
☐ 7 timer eller mer

12. Hvor lenge pleier du å holde på hver gang med disse aktivitetene? *(sett ett kryss for hver linje)*

	Mindre enn ½ time	½-1 time	Mer enn 1 time
•Ser på TV/DVD	☐	☐	☐
•Spiller PC/TV spill	☐	☐	☐
•Spiller, chatter eller surfer på nettet	☐	☐	☐

Om helse/helsetjenester

13. Har du funksjonsnedsettelse på noen av disse områdene?

	Nei	Litt	Middels	Mye
•Er bevegelseshemmet	☐	☐	☐	☐
•Har nedsatt syn *(selv om du evt bruker briller)*	☐	☐	☐	☐
•Har nedsatt hørsel	☐	☐	☐	☐
•Hemmet på grunn av kroppslig sykdom	☐	☐	☐	☐
•Hemmet på grunn av psykiske plager	☐	☐	☐	☐

•Bruker du rullestol: ☐Ja ☐Nei

14. Har du hatt kontakt med følgende hjelpetilbud SISTE ÅR? Hvis ja, kryss av for hvor ofte.

❑ **Spesialpedagogiske tiltak/spesialundervisning**
❍ Hver uke ❍ Hver måned ❍ Hver tredje måned ❍ Hvert halvår ❍ Sjeldnere

❑ **Pedagogisk psykologisk tjeneste (OT/PPT)**
❍ Hver uke ❍ Hver måned ❍ Hver tredje måned ❍ Hvert halvår ❍ Sjeldnere

❑ **Psykisk helsevern for voksne (VOP)**
❍ Hver uke ❍ Hver måned ❍ Hver tredje måned ❍ Hvert halvår ❍ Sjeldnere

15. Har, eller har du hatt:

Atopisk *(kløende)* eksem? ☐ Nei ☐ Ja, tidligere ☐ Ja, fortsatt

Høysnue? ☐ Nei ☐ Ja, tidligere ☐ Ja, fortsatt

Andre allergiske sykdommer? ☐ Nei ☐ Ja; tidligere ☐ Ja, fortsatt
Beskriv i så all:_____

Epilepsi? ☐ Nei ☐ Ja; tidligere ☐ Ja, fortsatt

Dren i ørene? ☐ Nei ☐ Ja; tidligere ☐ Ja, fortsatt

Nedsatt hørsel ☐ Nei ☐ Ja; tidligere ☐ Ja, fortsatt
Hvis fortsatt, kryss av behandling: ☐ Ingen ☐ Høreapparat ☐ Cochleaimplantat
☐ Døv, ingen apparater

Skjeling ☐ Nei ☐ Ja; tidligere ☐ Ja, fortsatt

Svekket syn ☐ Nei ☐ Ja; tidligere ☐ Ja, fortsatt;
Hvis fortsatt; ☐ Nærsynt ☐ Langsynt ☐ Blind ett øye ☐ Blind begge øyne
☐ Annet, beskriv;_____

Bruker briller? ☐ Nei ☐ Ja, hvilken styrke? _____

16. Har du

Fjernet falsk mandel (polypp, adenoid) ☐ Nei ☐ Ja
Fjernet mandlene? ☐ Nei ☐ Ja
Hatt feberkramper? ☐ Nei ☐ Ja, sist år gammel
Hatt hjernehinnebetennelse? ☐ Nei ☐ Ja
Hatt hodeskade med tap av bevissthet og innleggelse i sykehus? ☐ Nei ☐ Ja
Nedsatt førlighet i armer og/eller ben? ☐ Nei ☐ Ja, Beskriv i så fall:_____

17. Har, eller har du hatt, andre sykdommer som ikke er nevnt ovenfor?

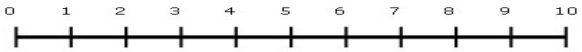
☐ Nei ☐ Ja; tidligere ☐ Ja, fortsatt

Beskriv i så fall:_____

18. I løpet av de siste 6 månedene: Hvor ofte har du hatt følgende plager? (Sett ett kryss for hver linje)

	Omtrent hver dag	Mer enn én gang i uken	Omtrent hver uke	Omtrent hver måned	Sjelden eller aldri
Hodepine	☐	☐	☐	☐	☐
Vondt i magen	☐	☐	☐	☐	☐
Vondt i ryggen	☐	☐	☐	☐	☐
Følt deg nedfor (trist)	☐	☐	☐	☐	☐
Vært irritabel eller i dårlig humør	☐	☐	☐	☐	☐
Nervøs	☐	☐	☐	☐	☐
Vanskelig for å sovne	☐	☐	☐	☐	☐
Svimmel	☐	☐	☐	☐	☐

19. I den grad du opplever smerte, hvordan vil du angi smerten på en skala fra 0-10? *(sett ring rundt det tallet som best uttrykker smerten)*



Ingen smerte — Verst tenkelig smerte

20. Stemmer noe av det som står nedenfor for deg? *(sett ett kryss for hver linje)*

	Stemmer	Stemmer ikke
•Smerter gjør det vanskelig for meg å sovne	☐	☐
•Smerter forstyrrer den gode nattesøvnen min	☐	☐
•Smerter gjør det vanskelig å sitte på arbeid	☐	☐
•Smerter gjør det vanskelig for meg å gå mer enn en kilometer	☐	☐
•På grunn av smerter har jeg problemer ved trening eller fysisk aktivitet	☐	☐

21. Har smertene alt i alt hindret deg i å utføre daglige aktiviteter? *(ett kryss pr. linje)*

- I arbeid
- I fritiden

Hvis ja, **hva slags smerter hindret deg i å utføre daglige aktiviteter?** *(sett eller flere kryss)*

Hodepine/migrene☐ Magesmerter☐ Muskel-/leddsmerter☐ Andre smerter☐

Om svangerskap

22. Har du og din partner noen gang prøvd å bli gravid? Ja ☐ Nei ☐

Hvis nei, gå til spørsmål **25**

23. Har du og din partner noen gang prøvd i mer enn ett år å bli gravid?

Ja ☐ Nei ☐

24. Hvor mange ganger har du og din partner i alt vært gravid?

☐ ganger

Om luftveiene (fra ISAAC)

25. Har du noen gang hatt tung pust eller piping/surkling/tetthet i brystet?

Ja ☐ Nei ☐

Hvis du har svart nei, gå til spørsmål **30**

26. Har du hatt tung pust eller piping/surkling/tetthet i brystet i løpet av de siste 12 månedene?

Ja ☐ Nei ☐

Hvis du har svart nei, gå til spørsmål **30**

27. Hvor mange anfall av tung pust eller piping/surkling/tetthet i brystet har du hatt i løpet av de siste 12 månedene?

☐ Ingen
☐ 1 til 3
☐ 4 til 12
☐ Mer enn 12
☐ Har slike plager hele tiden

28. Hvor ofte har din søvn i gjennomsnitt blitt forstyrret på grunn av tung pust eller piping/surkling/tetthet i brystet de siste 12 månedene?

☐ Aldri våknet
☐ Mindre enn 1 natt pr. uke
☐ 1 eller flere netter pr. uke

29. Har piping/surkling/tetthet i brystet eller tung pust vært så alvorlig de siste 12 månedene at du har hatt problemer med å snakke slik at du bare kunne si ett eller to ord mellom hver pust? Ja ☐ Nei ☐

30. Har du noen gang hatt astma? Ja ☐ Nei ☐

31. Har du i løpet av de siste 12 månedene hatt tung pust eller piping/surkling/tetthet i brystet under eller etter fysisk trening, aktiv lek eller mosjonering? Ja ☐ Nei ☐

32. Har du i løpet av de siste 12 månedene hatt tørr hoste om natten, utenom hoste i forbindelse med en forkjølelse eller andre luftveisinfeksjoner? Ja ☐ Nei ☐

33. Opplever du pustebesvær utover normal andpustethet i forbindelse med anstrengelse? Ja ☐ Nei ☐

Dersom ja:
Er pustebesværet verst *under* anstrengelse eller *rett etter* anstrengelse:
☐ under ☐ rett etter ☐ vet ikke

Er pustebesværet verst på *ut-pust* eller *inn-pust*:
☐ ut-pust ☐ inn-pust ☐ vet ikke

Er pustebesværet ledsaget av *smerter i brystet*:
☐ nei ☐ ja

34. Er hoste eller tung pust *etter* anstrengelse/trening et problem for deg? Ja ☐ Nei ☐

35. Har du diagnosen anstrengelses-utløst astma? Ja ☐ Nei ☐

36. Har du pusteproblemer ut over det normale ved vanlig fysisk anstrengelse?

☐ Nei ☐ Litt mer enn normalt ☐ Mye mer enn normalt

37. Lager du "skrapelyder" eller andre unormale lyder fra strupen ved fysisk anstrengelse?

☐ Nei ☐ Litt ☐ Mye

Spørsmål om stemmen din

38. Er stemmen din mer hes enn hos andre på samme alder?

☐ Ikke i det hele tatt ☐ Litt ☐ Moderat ☐ Mye mer ☐ Ekstremt

Om bruk av medisiner

39. Har du brukt astma-medisin i forbindelse med trening eller anstrengelse i løpet av de siste 12 månedene?

Ja ☐ Nei ☐

40. Har du brukt medisiner mot astma i løpet av de siste 12 månedene?

☐ Nei
☐ Ja, av og til
☐ Ja, regelmessig i mer enn 3 måneder

41. Dersom du har brukt medisiner mot astma i løpet av de siste 12 månedene, angi type medisiner: *(kryss for de typene du har brukt - du kan krysse flere steder. De mest brukte angis med navn i parentes)*

☐ Medisiner som åpner luftrørene *(Bricanyl, Ventoline, Airomir, Oxis, Serevent)*
☐ Kortison til inhalasjon *(Pulmicort, Becotide, Aerobec, Flutide, Alvesco)*
☐ Medisin som både åpner og forebygger *(Symbicort, Seretide, Relvar)*
☐ Singulair tabletter (*Montelukast*)
☐ Lomudal til inhalasjon

42. Hvor ofte har du brukt reseptfrie medisiner mot følgende plager i løpet av de siste 3 månedene? *(sett ett kryss pr. linje)*

	Sjelden /aldri	1-3g /uke	4-6g /uke	Dag-lig
•Hodepine	☐	☐	☐	☐
•Muskel-/leddsmerter	☐	☐	☐	☐
•Magesmerter	☐	☐	☐	☐
•Ryggsmerter	☐	☐	☐	☐
•Andre plager	☐	☐	☐	☐

43. Bruker du medisiner som du har fått av lege på resept? Ja ☐ Nei ☐

Om kosthold og spisevaner

44. Står du på noen diett ordinert av lege?

Ja	Nei
☐	☐

Hvis Ja, spesifiser:_____

45. Hvor ofte spiser du vanligvis disse måltidene? *(sett ett kryss pr. linje)*

	Hver dag	Oftere enn 3 dager i uken	Sjeldnere enn 3 dager i uken	Aldri
•Frokost	☐	☐	☐	☐
•Matpakke/formiddagsmat	☐	☐	☐	☐
•Middag	☐	☐	☐	☐
•Kveldsmat	☐	☐	☐	☐

46. Nedenfor er en liste over ting som gjelder spisevaner. Kryss av for hva som passer deg. *(sett ett kryss pr. linje)*

	Aldri	Sjelden	Ofte	Alltid
•Når jeg først har begynt å spise, kan det være vanskelig å stoppe	☐	☐	☐	☐
•Jeg bruker for mye tid til å tenke på mat	☐	☐	☐	☐
•Jeg føler at maten kontrollerer livet mitt	☐	☐	☐	☐
•Når jeg spiser, skjærer jeg maten opp i små biter	☐	☐	☐	☐
•Jeg bruker lengre tid enn andre på et måltid	☐	☐	☐	☐
•Eldre mennesker synes at jeg er for tynn	☐	☐	☐	☐
•Jeg føler at andre presser meg til å spise	☐	☐	☐	☐
•Jeg kaster opp etter at jeg har spist	☐	☐	☐	☐

47. Hvordan vurderer du din egen vekt?

☐	☐	☐	☐	☐	☐
Svært undervektig	Svært undervektig	Litt undervektig	Passelig	Litt overvektig	Svært overvektig

48.

	Stemmer helt	Stemmer delvis	Stemmer ikke
Jeg er fornøyd med spisevanene mine	☐	☐	☐
Jeg trøstespiser	☐	☐	☐
Jeg har skyldfølelse i forbindelse med spising	☐	☐	☐
Jeg må ha strenge dietter for å kontrollere hvor mye jeg spiser	☐	☐	☐
Jeg synes jeg er for tykk	☐	☐	☐

49. Hvor ofte spiser/drikker du vanligvis noe av følgende? *(sett ett kryss pr linje)*

	Hver dag	Oftere enn 3 dager i uken	Sjeldnere enn 3 dager i uken	Aldri
•Grønnsaker	☐	☐	☐	☐
•Frukt	☐	☐	☐	☐
•Brødmat	☐	☐	☐	☐
•Melk	☐	☐	☐	☐
•Kjøtt	☐	☐	☐	☐
•Fisk	☐	☐	☐	☐
•Godteri	☐	☐	☐	☐
•Cola/brus/saft med sukker	☐	☐	☐	☐
•Cola/brus/saft uten sukker	☐	☐	☐	☐
•Fastfood (pizza, pølse, burger)	☐	☐	☐	☐

Om tobakk og alkohol

50. Har du prøvd å røyke? *(minst en sigarett)* Ja ☐ Nei ☐

Hvis nei, gå til spørsmål **52**

51. Røyker du? *(sett ett kryss og oppgi evt. antall sigaretter)*

☐ Ja, jeg røyker ca _____ sigaretter daglig
☐ Ja, jeg røyker av og til, men ikke daglig
☐ Nei, ikke nå, men tidligere røykte jeg av og til
☐ Nei, ikke nå lenger, men tidligere røykte jeg ca. _____sigaretter daglig
☐ Nei, jeg røyker ikke

52. Hvis du røyker eller har røykt daglig, hvor gammel var du da du begynte å røyke? _____år gammel

53. Bruker du eller har du brukt snus eller lignende? *(sett ett kryss)*

☐ Nei, aldri ☐ Ja, men jeg har sluttet ☐ Ja, av og til ☐ Ja, hver dag

54. Hvis du bruker eller har brukt snus, hvor gammel var du da du begynte med snus?
_____år gammel

	Ja	Nei
55. Drikker du alkohol?	☐	☐

Hvis ja,

56. Omtrent hvor mye øl, cider, rusbrus, vin eller brennevin drikker du vanligvis i løpet av to uker? Regn ikke med alkoholfritt øl.

	antall
Øl - flasker (0,33 dl):	
Cider/ Rusbrus (ca 0,33 dl):	
Vin - antall glass (ca 1,5 dl):	
Brennevin - antall glass (ca 0,4 dl):	
Hjemmebrent - antall glass (ca 0,4 dl):	

Om søvn

	Stemmer helt	Stemmer delvis	Stemmer ikke
57. Jeg har problemer med innsovning og/eller våkner ofte	☐	☐	☐

Dersom stemmer helt/ stemmer delvis:

58. Hvor lenge har du hatt disse vanskene?

59. Hvor mange netter i uken har du:

	0	1	2	3	4	5	6	7
- innsovningsvansker.	☐	☐	☐	☐	☐	☐	☐	☐
- våkner ofte.	☐	☐	☐	☐	☐	☐	☐	☐

	Stemmer helt	Stemmer delvis	Stemmer ikke
60. Jeg snorker (eller andre sier jeg snorker)	☐	☐	☐

	Stemmer helt	Stemmer delvis	Stemmer ikke
61. Kjenner du deg søvnig eller trøtt om dagen?	☐	☐	☐

Dersom stemmer helt/stemmer delvis:

62. Hvor mange dager i uken opplever du

	0	1	2	3	4	5	6	7
- søvnig (jeg dupper lett av).	☐	☐	☐	☐	☐	☐	☐	☐
- trøtt (er sliten/uopplagt).	☐	☐	☐	☐	☐	☐	☐	☐

	Hverdager	Helg
63. Når legger du deg vanligvis?		
64. Når står du vanligvis opp?		

65.

	Timer	Minutter
Hvor lang tid går det vanligvis fra du legger deg til du sovner?		
Hvor lenge er du våken i løpet av natten (etter at du først har sovnet)?		
Hvor mye søvn trenger du for å føle deg uthvilt?		

66.

	Aldri	Sjeldent (noen ganger per år)	Iblandt (noen ganger per måned)	For det meste (flere ganger i uken)	Alltid (hver dag)
Hvor ofte tar du deg en blund på dagtid?	☐	☐	☐	☐	☐
Hvor ofte forsover du deg til arbeid?	☐	☐	☐	☐	☐

67. Hvor mange av de viste elektroniske gjenstandene benytter du på soverommet den siste timen før du sovner? Sett kryss.

___ PC
___ Mobiltelefon
___ MP3-spiller
___ Nettbrett
___ Spillekonsoll (Playstation, Xbox, WII etc)
___ TV

Takk for din deltakelse!

Appendix II

HELSE BERGEN
Haukeland universitetssjukehus

Foreldreskjema

Etter undersøkelse, barn født etter svangerskap komplisert av tidlig fostervannsavgang

Skjemaet skal fyllast ut av pårørande og gjevast til legen. Legen vil i tillegg fylle ut eit skjema om barnets utvikling, og eventuelle sjukdomar og funksjonshemningar

Barnets navn _____ fødselsdato _____

Mors navn _____ fødselsnummer _____

Adresse _____ postnummer _____ stad _____

Næraste pårørande (dersom forskjellig frå mor) _____ (evt slektskap)_____

1. Har barnet vore innlagt i sjukehus etter 2 års alder? Ja/nei i tilfelle kor mange gongar _____
2. dersom barnet har vore innlagt i sjukehus **etter 2 års alder skriv årsak og kor gammalt barnet var**

Opphald nummer	årsak til innleggelse	Alder i heile år	

Spørsmål om lungefunksjonen

3 Har barnet nokon gang etter nyføddperioden hatt tung pust, piping, surkling eller tetthet i brystet (kryss av for det alternativet du meiner passer best

Ja __ dersom ja

__ berre før, men ikkje etter 2 års alder

__ både før og etter 2 års alder

__ berre etter 2 års alder

nei __ (dersom nei, gå til spørsmål 8)

4. har barnet hatt tun g pust eller piping, surkling eller tetthet i brystet i løpet av dei siste 12 månadene?
   Ja __
   Nei __ dersom nei gå til spørsmål 8
5. kor mange anfall med tung pust eller piping, surkling eller tetthet i brystet har barnet hatt i løpet av dei siste 12 månadene?
   a. Ingen __
   b. 1-3 __
   c. 4-12 __
   d. Meir enn 12 __
   e. Har slike plager heile tida __
6. kor ofte har barnets søvn i gjennomsnitt blitt forstyrra på grunn av piping, surkling eller tetthet i brystet dei siste 12 mndr?
   a. Aldri vakna __
   b. Mindre enn ei natt/veke __
   c. 1 eller fleire netter/veke __
7. har piping, surkling, tetthet i brystet eller tung pust vore så alvorlig dei siste 12 mndr at barnet har hatt problem med å snakke slik at han/ho berre kunne seie eitt eller to ord mellom kvar pust?
   a. Ja __
   b. Nei
      __

8. har barnet nokon gang hatt astma?
   a. Ja __
   b. Nei __
9. har barnet i løpet av dei siste 12 mndr hatt tung pust eller piping, surkling, tetthet i brystet under eller etter fysisk trening, aktiv leik eller mosjonering? Ja __ nei __
10. har barnet i løpet av dei siste 12 mndr hatt tørr hoste om natta, utenom hoste i forbindelse med forkjølelse eller andre luftvegsinfeksjonar?
    a. Ja __
    b. Nei __
    c. Heile tida __
11. har barnet brukt oksygen (surstoff) heime etter 2 års alder?
    a. Nei __
    b. Ja, men ikkje no lenger __
    c. Ja, bruker det fortsatt, i alle fall i periodar __
12. har barnet brukt astmamedisinar etter 2 års alder? (antibiotika/penicillin ved lungebetennelse blir ikkje rekna med)
    a. nei, barnet har ikkje brukt medisinar __
    b. ja, barnet brukte medisinar tidligare etter 2 års alder, men ikkje lenger __
    c. ja, barnet bruker fortsatt medisinar, i så fall kva medisinar __
        i. inhalasjonssteroidar (Flutide, Pulmicort, Seretide, Symbicort, i så fall fast eller i periodar __
            1. fast daglig __
            2. berre i periodar med forkjølelse/tung pust __
        ii. anfallsmedisinar (Ventolin, Bricanyl, Airomir, Serevent)
            1. fast daglig __
            2. berre ved tung pust eller før anstrengelse __
        iii. Singulair
        iv. Andre lungemedisinar __ (skriv ned kva medisinar _____)
13. har barnet hatt episodar med lungebetennelse eller bronkitt som har blitt behandla med penicillin eller andre antibiotika etter 2 års alder?
    a. Nei __
    b. Ja __ i så fall:
        i. Om lag kor mange gangar fram til for 1 år tilbake __ gangar
        ii. Om lag kor mange gangar siste år __ gangar
14. kor mange gangar har barnet fått penicillin eller andre antibiotika for andre sjukdomar enn lungebetennelse etter 2 års alder? Skriv 0 dersom ingen __

Spørsmål om andre sjukdomar

15. har, eller har barnet hatt atopisk (kløande) eksem nei __ ja, tidlegare __ ja, fortsatt __
16. har, eller har barnet hatt, høysnue nei __ ja, tidlegare __ ja, fortsatt __
17. har, eller har barnet hatt, andre allergiske sjukdomar nei __ ja, tidlegare __ ja, fortsatt __ beskriv i så fall:
18. har, eller har barnet hatt, dren i ørene nei __ ja, tidlegare __ ja, fortsatt __
19. har barnet fått fjerna falske mandlar (polypp, adenoid) nei__ ja __
20. har barnet fått fjerna mandlane nei__ ja __
21. har, eller har barnet hatt, andre sjukdomar som ikkje er nemnt ovanfor nei __ ja, tidlegare __ ja, fortsatt __
    Beskriv i så fall _____

Spørsmål om ernæring

22. Korleis vil du beskrive kor flink barnet har vore til å spise frå 2 års alder?
    a. Ikkje spisevanskar av betydning __
    b. Spisevanskar før, men ikkje siste år __
    c. Heile tida spisevanskar, også no __
23. Dersom barnet har eller har hatt spisevanskar etter 2 års alder, korleis har desse arta seg (kryss for alle aktuelle)
    a. Spiser lite, vanskelig å få ti l å spise (småspist) __
    b. Vanskar med å spise/svelge klumpar og fast mat __
    c. Liker berre enkelte ting, kva vil han/ho ikkje spise __
    _____
    d. Andre spisevanskar __ beskriv _____

Spørsmål om aktivitet, ferdigheter og utvikling

24. Kor uthaldande er barnet i leik og aktivitet?
    a. __ held følgje med jamaldrande born i leik og aktivitet
    b. __ litt mindre uthaldande enn jamaldrande born
    c. __ mykje mindre uthaldande enn jamaldrande born
25. korleis oppfattar du barnets fysiske ferdigheter (grovmotorikk) (for eksempel løping, hopping, sparke ball, sykling osv)
    a. __ meir "kløne te" eller umoden i sine ferdigheter
    b. __ lik jamaldrande

c. __ flinkare enn dei fleste jamaldrande

26. korleis oppfatter du barnets ferdigheter med hendene (for eksempel teikne, klippe, bygge med Lego osv)
    a. __ meir "kløhete" eller umoden i sine ferdigheter
    b. __ lik jamaldrande
    c. __ flinkare enn dei fleste jamaldrande
27. korleis vil du beskrive barnets språk i dag (velg det alterantivet du synest passer best
    a. __ barnet snakker like godt som jamaldrande born
    b. __ Barnet har same ordforråd som andre, men dårlegare uttale
    c. __ Barnet har mindre ordforråd, men god uttale
    d. __ Barnet har både mindre ordforråd og dårlegare uttale
    e. __ Barnet har ikkje, eller har svært lite, språk
28. har barnet sidan 2 års alder hatt behov for spesielle hjelpetiltak som kontakt med (kryss alle aktuelle)
    a. __ fysioterapeut
    b. __ logoped
    c. __ ekstra støttetiltak i barnehagen
    d. __ PPT (pedagogisk psykologisk teneste)
    e. __ psykolog/psykiater
    f. __ barne- og ungdomspsykiatri
    g. __ habiliteringsteneste
29. har barnet gått i barnehage sidan 2 års alder
    a. __ nei
    b. __ i barnehage tidlegare, men ikkje no, kor mange år i barnehage __ år
    c. __ går fortsatt i barnehage, og har gått i kor mange år ___
30. korleis fungerer barnet saman med andre born, for eksempel i barnehagen
    a. __ barnet skiljer seg ikkje frå andre jamaldrande born
    b. __ barnet har samspelvanskar med andre born (dersom samspelvanskar, angi korleis (leire rubrikkar kan kryssast)
        i. __ barnet blir plaga av andre born, føler seg utanfor og sky
        ii. __ barnet er aggressivt, urolig og plager andre born
        iii. __ barnet mistrivst, føler seg utanfor og isolert utan å bli plaga eller plagar andre
    c. __ andre vanskar i samspel med andre, beskriv desse _____

Spørsmål om søvn

31. har barnet etter 2 års alder hatt søvnvanskar
    a. __ ingen søvnvanskar desse åra
    b. __ søvnvanskar tidlegare, ikkje siste år
    c. __ fortsatt søvnvanskar
32. dersom barnet har hatt søvnvanskar etter 2 års alder eller fortsatt har søvnvanskar, korleis vil du beskrive desse (fleire rubrikkar kan kryssast av)
    a. __ vanskar med å legge seg til å sove om kvelden
    b. __ vaknar i løpet av natta
    c. __ vaknar uvanlig tidleg
    d. __ vaknar uvanlig seint
    e. __ andre søvnvanskar, beskriv _____

Avføring og vannlatning

33. tissar barnet på seg om dagen? __ nei __ av og til __ ofte
34. tissar barnet på seg om natta? __ nei __ av og til __ ofte
35. får barnet avføring i bukse/bleie om dagen? __ nei __ av og til __ ofte
36. får barnet avføring i bukse/bleie om natta? __ nei __ av og til __ ofte
37. Kor ofte har barnet avføring?
    a. Meir enn 2 ggr pr dag __ 1-2 ggr/dag __ sjeldnare __
38. korleis er avføringa? __ normal forma __ laus __ hard

Litt om familien

39. Kor mange søsken eller halvsøsken har barnet? ___ søsken/halvsøsken
    For heilsøsken: oppgje alder, kjønn, høgde og vekt:
    Søsken nr 1: ____ år og ____ mndr, gut __ jente ___ høgde _____cm vekt _____ kg
    Søsken nr 2: ____ år og ____ mndr, gut __ jente ___ høgde _____cm vekt _____ kg
    Søsken nr 3: ____ år og ____ mndr, gut __ jente ___ høgde _____cm vekt _____ kg
    Søsken nr 4: ____ år og ____ mndr, gut __ jente ___ høgde _____cm vekt _____ kg
    Søsken nr 5: ____ år og ____ mndr, gut __ jente ___ høgde _____cm vekt _____ kg

40. foreldra sin høgde og vekt
    a. mors høgde _____ cm vekt _____ kg
    b. fars høgde _____ cm vekt _____ kg

41. kven bur barnet saman med til dagleg
    a. ___ mor og far
    b. ___ berre mor
    c. ___ berre far
    d. ___ både mor og far, men kvar for seg (for eksempel ei veke hos kvar)
    e. ___ mor og ny partnar (stefar)
    f. ___ far og ny partnar (stemor)
    g. ___ forsterforeldre
    h. ___ andre (kven) _____
42. spørsmål om spesielle sjukdomar i familien
    a. Har, eller har nokon hatt, astma: ___ ingen ___ mor ___ far ____ søsken
    b. Har, eller har nokon hatt, høysnue: ___ ingen ___ mor ___ far ____ søsken
    c. Har eller har nokon hatt, atopisk eksem: ___ ingen ___ mor ___ far ____ søsken
    d. Har, eller har nokon hatt, åtferdsvanskar, vanskar med konsentrasjon, lærevanskar (ADHD ol)
       ___ ingen ___ mor ___ far ____ søsken
43. Røykjer foreldre eller omsorgspersonar (kryss alle aktuelle)
    a. __ nei, verken mor, far eller andre omsorgspersonar
    b. __ ja, mor
    c. __ ja, far
    d. __ Ja, sambuar av mor eller far
    e. __ ja, andre som bur i huset
44. blir det røykt inne i huset __ nei __ ja
45. Kva er høgaste fullførte utdanning for mor og far

    Mor
    a. __ mindre enn 9-årig skule
    b. __ 9-årig skule (ungdomsskule)
    c. 9-årig skule + 1-2 års vidaregåande skule
    d. 9-årig skule + 3 års vidaregåande skule (inkl gymnas)
    e. Høgare utdanning, for eksempel distriktshøgskule, sykepleiarskule, lærarhøgskule
    f. Høgare utdanning på universitetsnivå

    Har mor norsk som morsmål: __ ja __ nei dersom nei, kva språk _____

    Far
    g. __ mindre enn 9-årig skule
    h. __ 9-årig skule (ungdomsskule)
    i. 9-årig skule + 1-2 års vidaregåande skule
    j. 9-årig skule + 3 års vidaregåande skule (inkl gymnas)
    k. Høgare utdanning, for eksempel distriktshøgskule, sykepleiarskule, lærarhøgskule
    l. Høgare utdanning på universitetsnivå

    Har far norsk som morsmål: ___ ja __ nei dersom nei, kva språk? _____
46. kva er mors og fars yrkesmessige situasjon?

    Mor
    a. __ fulltidsarbeidande (minst 30 t/veke)
    b. __ Deltidsarbeidande (mindre enn 30 t/veke)
    c. __ arbeidsledig/på tiltak/abeidssøkande
    d. __ student/elev
    e. __ heimearbeidande
    f. __ trygda/under attføring
    g. __ anna
    h. __ mors yrke _____

    Far
    a. __ fulltidsarbeidande (minst 30 t/veke)
    b. __ Deltidsarbeidande (mindre enn 30 t/veke)
    c. __ arbeidsledig/på tiltak/abeidssøkande
    d. __ student/elev
    e. __ heimearbeidande
    f. __ trygda/under attføring
    g. __ anna
    h. __ fars yrke _____

Legen vil gje utfyllande kommentarar om barnets helse og utvikling på eige skjema
Vi takkar for at du har vore villig til å delta i undersøkelsen om barn som er født etter svangerskap komplisert av svært for tidlig fostervannsavgang si utvikling og helse. Vi meiner at ein slik undersøkelse er viktig for å forstå meir kva dette betyr for barnet seinare i livet

Dersom du har tilleggsopplysningar/kommentarar er det fint om du skriv det på eige ark og legge ved spørjeskjemaet

Appendix III

• HELSE BERGEN
Haukeland universitetssjukehus

Barn fødd etter svangerskap komplisert av langvarig fostervannsavgang

Barnets navn ---------- fødselsdato _____

Mars navn _____

Adresse _____ postnummer _____ stad _____

Mars fødselsnummer

Dersom tvilling eller meir, kva nummer i rekka _

Dato for unders0kelse _____

1. vest og fysiologi vekt __ kg, høgde ___ cm hodeomkrets cm

blodtrykk (sitjande, lavast av mållingar) systolisk __ diastolisk

2. auge/syn (angi alle aktuelle felt ut fra kjennskap og klinisk vurdering)
   a. normal
   b. strabisme, dersom ja _ operert _ ikkje-operert
   c. _ myopi, anf0r grad dersom kjent
   d. _ Hypermetropi, anf0r grad dersom kjent _____
   e. _ astigmatisme
   f. _ andre synsdefektar, anf0r _____
   g. _ blind, arsak _____
   h. Har barnet vore hos 0yelege i l0pet av siste 2 ar _ja nei
   i. Bruker barnet briller? _ja nei

3. h0rsel (anfor alle aktuelle felt ut fra kjennskap og klinisk vurdering)
   a. _ normal hørsel begge 0yrer
   b. _ normal hørsel ett av 0yrene
   c. _ hørselsvekkelse - treng ikkje h0reapparat
      i. Type: _ nevrogent konduktivt blanda _ukjent
   d. _ hørselsvekkelse - treng h0reapparat
      Type: _ nevrogent konduktivt blanda _ukjent
   e. døv
      i. Type: _ nevrogent konduktivt blanda _ ukjent
   f. Kokleaimplantat nei ja
   g. Har barnet vore formelt hørselstesta etter 2 ars alder
      i. _ pa helsestasjon
      ii. _ pa h0resentral
      iii. nei

4. nevrologisk vurdering (fyll ut alle relevante)
   a. _ normal nevrologisk status
   b. _ forsinka eller umoden motorikk, ikkje CP
   c. _ spastisk diplegi utan affeksjon av overekstremitetar
   d. _ spastisk diplegi, ogsa med affeksjon av overekstremitetar
   e. _ spastisk hemiplegi
   f. _ spastisk kvadriplegi
   g. atetotisk CP eller tonusvekslar
   h. _ hydrocephalus, i sa fall_ drenbehandla _ ikkje drenbehandla
   i. _ andre nevrologiske tilstandar, beskriv _____

5. Mental utvikling (atferd, konsentrasjon, kognitlve ferdigheter vurdert frå anamnese og klinisk undersøkelse)
   a. 5amarbeider og konsentrerer barnet seg om oppg3ver pa aldersadekvat mate _ja nei
      1. dersom ja, er dette _ observert bed0mt fr3 anamnese
   b. har barnet generelt forsinka ferdigheter? _ja nei
      1. dersom ja, er dette _ observert bed0mt fr3 anamnese
   c. er barnet ukonsentrert og urolig? ja nei
      1. dersom ja, er dette _ observert bed0mt fr3 anamnese
   d. _ andre anmerkningar _____

6. andre nevrologiske sjukdomar?(fyll ut alle aktuelle)

a. _ ingen
b. _ epilepsi, type _____ antiepileptika: _____
c. anna. Anf0r _____

7. lunger (fyll ut alle aktuelle)
a. _ ingen klinisk patologi
b. _ astma (dvs periodevis obstruktivitet/kronisk hoste
c. _ kronisk auka tr0ttbarhet vurdert som pulmonalt betinga, BPD-sekvele som ikkje har astmatisk preg (kroniske vedvarande lungesymptom)

8. grad av lungesymptom (grad 1-5)
a. _ ingen lungesymptom ut over vanlege forkj0lelsessymptom {utan steroidar)
b. _ Grad 1, <5 episodar med obstr arlig, varighet < 1 veke kvar gang, elles normal lungefunksjon (utan steroidar) korte episodar med anstr uttøst obstruktivitet somraskt blir oppheva me beta-stimulator
c. _ grad 2 5-10 episodar med obstruktiv årleg (utan vedl hold steroidar varighet < 1 veke
d. _ grad 3 > 10 episodar med obstr arlig, symptom og nedsatt aktivitet < 1 veke kvar gang, eller meir langvarige periodar (totatt 4 mndr / ar) med obstr eller nedsatt lungefunksjon lange symptomfrie periodar med normal lungefunksjon innimellom astma med symptom pa grad 1-2 som tar steroidar som vedlikehald blir sett til grad 3. ved symptom og teikn pa obstruksjon eller "stum" astma i > 4 mndr arlig set til grad 4
e. _ grad 4 > S episodar med langvarig obstruksjon med nedsett lungefunksjon i 6 mndr '3rlig eller meir. Symptom til grad 3 men tar steroidar kontinuerlig set ti1 grad 4
f. _ grad S kronisk funksjonshemmande obstruktivitet eller restriktiv lungesjukdom med alvorlig forverrelse trass kontinuerlig bruk av medisinar som inhalasjonssteroidar. Obstruktivetet med symptom til grad 3 eller 4 der det krevst h0ge vedl holdsdosar inhal steroidar eller periodevis perorale steroidar set til grad 5.

9. medikament for lungesjukdom no:
a. _ingen
b. _ inhalasjonssteroidar _ periodevis {for eksempel forkj0l) _ kontinuerlig preparat_. d0gndose __ ug
c. _ kombinasjonspreparat (td Seretide) _ periodevis (td forkj0lelse) _ kontinuerlig preparat____ d.dose __ _
d. _ Beta2 stimulator _ daglig _ fleire ggr/veke _ ca 1 gang/veke _ sjeldnare
e. _ Singulair
f. andre medikament beskriv _____

10. hjerteproblem
a. _ingen
b. _ cor pulmonale, i fall _ asymptomatisk __ hjertesvikt
c. _ andre hjerte/sirkulasjonsproblem, beskriv _____
d. _ bruker hjertemedsinar beskriv _____

11. 0vre tuftvegar
a. _ ingenplager
b. _ periodevis strider i svelg, larynx eller trakea
c. trakealstenose
d. _ dysfoni, svak eller hes stemme
e. trakeostomi
f. _ andre sjukdomar, beskriv _____

12. behandling 0vre luftvegar (kryss alle aktuelle)
a. _ ingen intervensjonar
b. utf0rt adenotomi
c. utf0rt tonsillektomi
d. _ utf0rt drensinnleggelse 0rer
e. _ andre inngrep beskriv _____

13. tenner (fyll ut a11e aktuelle)
a. normale forhold
b. _ emaljedefektar
c. _ feil ved tannstilling
d. _ forandringar av fortenner som kan skuldast endotrakealtubar eller sonde
e. karies, dersom ja: inntil 3 hot fleire enn 3 hol

14. ford0yelsesorgan (fyU ut alle aktuelle)
a. _ ingen patologi
b. barnet har hatt GErefluks etter 2 ars alder, dersom ja, basert pa
_ klinikk + evt radiologisk us
ii. _ 24 t pH-maling
l. dersom ja, har barnet tatt behandling? Ja nei
2. har barnet fortsatt G0R-plager ja nei
3. dersom ja, tar barnet fortsatt behandling ja nei
c. _ svelgvanskar, dysfagi, beskriv _____

d. _malabsorbsjon, anf0r arsak grad _____

e. __ gastrostomi/PEG

f. andre unormale forhold, besknv _____

15. nyrer/urinvegar

a. _ ingen patologi

b. _ nedsatt nyrefunksjon,årsak/grad _____

c. _annan patotogi _____

16. atopi/allergi

a. _ ingen patologi

b. _ har eller har hatt atopisk eksem

c. _ har gastrointestinal allergi

d. _ har allergisk rhinokonjunktivitt

i. Utf0rt prikktest	nei	_ ja, negativ	_ja, positiv
ii. Utf0rt total s-lgE	nei	_ja, normal	_ja, forh0ya
iii. Utf0rt spesifikk lgE	nei	_ja, normal	_ja, forh0ya

17. andre organsystem

a. _ ingen patologi

b. _ patologi, diagnoser _____

18. undersøkjar si vurdering av barnet i undersøkelsessituasjonen

	1 2 3 4 5 6 7	
a. oppmerksomhet		
ekstremt konsentrert om oppgave	_____	svært uoppmerksom
ikkje distraherbar	_____	lett distraherbar
b. utf0ring av unders0kelse		
svært lavt aktivitetsniv3	_____	h0gt aktivitetsniva
c. emosjonell tilstand		
sosialt svært tillitsfull	_____	sosialt svært usikker
overdreven tru på eigne ferdigheter	_____	inga tru pa eigne ferdigheter
d. kommunikasjon		
f3r raskt god kontakt	_____	svcært vanskelig a ta kontakt
e. språk		
svært god artikulasjon	_____	svært d3rlig artikulasjon
svært godt ekspressivt spriik	_____	svært d3rleg ekspressivt spriik
sært god ordforstaelse	_____	svært diirleg ordforst3else
f. generell bed0mmelse av testutf0relse		
barnet utf0rte til sitt optimale	_____	barnet brukte lite av sitt potensiale

lege som gjorde undersøkelse: _____

Papers I, II, and I

Development of lung diffusion to adulthood following extremely preterm birth

Emma Satrell[1,2], Hege Clemm [1,3], Ola Drange Røksund [3,4], Karl Ove Hufthammer [5], Einar Thorsen[1], Thomas Halvorsen [1,3] and Maria Vollsæter[1,3]

[1]Dept of Clinical Science, University of Bergen, Bergen, Norway.[2]Dept of Pediatric and Adolescent Medicine, Drammen Hospital, Vestre Viken Hospital Trust, Drammen, Norway.[3]Dept of Pediatric and Adolescent Medicine, Haukeland University Hospital, Bergen, Norway.[4]Faculty of Health and Social Sciences, Western Norway University of Applied Sciences, Bergen, Norway.[5]Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway.

Corresponding author: Emma Satrell (emma.satrell@uib.no)

Shareable abstract (@ERSpublications)
Pulmonary diffusing capacity following extremely preterm (EP) birth was reduced compared with term-born subjects. From mid-childhood to adulthood, development tracked in parallel in the EP and term-born groups, with preterms following lower trajectories. https://bit.ly/3ARPD7D

Cite this article as: Satrell E, Clemm H, Røksund OD, et al. Development of lung diffusion to adulthood following extremely preterm birth. Eur Respir J 2022; 59: 2004103 [DOI: 10.1183/13993003.04103-2020].

Received: 6 Nov 2020
Accepted: 21 Sept 2021

Abstract

Background Gas exchange in extremely preterm (EP) infants must take place in fetal lungs. Childhood lung diffusing capacity of the lung for carbon monoxide (D_{LCO}) is reduced; however, longitudinal development has not been investigated. We describe the growth of D_{LCO} and its subcomponents to adulthood in EP compared with term-born subjects.

Methods Two area-based cohorts born at gestational age ⩽28 weeks or birthweight ⩽1000 g in 1982-1985 (n=48) and 1991-1992 (n=35) were examined twice, at ages 18 and 25 years and 10 and 18 years, respectively, and compared with matched term-born controls. Single-breath D_{LCO} was measured at two oxygen pressures, with subcomponents (membrane diffusion (D_M) and pulmonary capillary blood volume (V_C)) calculated using the Roughton-Forster equation.

Results Age-, sex- and height-standardised transfer coefficients for carbon monoxide (K_{CO}) and D_{LCO} were reduced in EP compared with term-born subjects, and remained so during puberty and early adulthood (p-values for all time-points and both cohorts ⩽0.04), whereas alveolar volume (V_A) was similar. Development occurred in parallel to term-born controls, with no signs of pubertal catch-up growth nor decline at age 25 years (p-values for lack of parallelism within cohorts 0.99, 0.65, 0.71, 0.94 and 0.44 for z-D_{LCO}, z-V_A, z-K_{CO}, D_M and V_C, respectively). Split by membrane and blood volume components, findings were less clear; however, membrane diffusion seemed most affected.

Conclusions Pulmonary diffusing capacity was reduced in EP compared with term-born subjects, and development from childhood to adulthood tracked in parallel to term-born subjects, with no signs of catch-up growth nor decline at age 25 years.

Introduction

Extremely preterm (EP) infants (born before 28 weeks of pregnancy) currently account for one in 200 live births in high-income countries [1], with survival approaching 90% for infants born at 27 weeks gestation [2]. EP birth requires that fetal lungs develop in an extra-uterine environment while providing gas exchange for the newborn individual. The lungs at this stage have no proper gas exchanging units, as alveolarisation has hardly commenced [3, 4]. Lifesaving intensive care is required and relies on measures that are harmful to developing lungs, such as positive pressure ventilation and hyperoxia. The pulmonary complication of this scenario is labelled bronchopulmonary dysplasia (BPD) [5]. The few autopsy studies that have been published from infants who have died with BPD reveal “acinar dysplasia”, characterised by fewer and larger alveoli, and thickened alveolar-capillary membranes [6, 7]. We do not know how these

structural injuries evolve later in life, but recent magnetic resonance imaging (MRI) studies suggest continued alveolar development until adolescence [8].

The standard functional measure of alveolar gas exchange is diffusing capacity of the lung for carbon monoxide (D_{LCO}) [9]. D_{LCO} is a compound measure reflecting lung volumes, surface area accessible for gas exchange, thickness of the alveolar-capillary barrier and pulmonary capillary blood volume. By using two different oxygen pressures during measurements, D_{LCO} can be split into two components: transfer across the alveolar-capillary membrane (D_M) and the rate of reaction with haemoglobin, reflecting the pulmonary capillary blood volume (V_C) [10].

Studies report reduced D_{LCO} in EP children and adolescents, suggesting persistent deficits of acinar function [11-15], although with surprisingly little influence from BPD [13, 15, 16]. Airway versus blood vessel interactions during lung development are poorly understood, and the relative impact from D_M and V_C for D_{LCO} is therefore of interest [14, 17-19]. We aimed to test the hypothesis that impaired D_{LCO} in EP subjects persists over time, without age-related catch-up or decline when compared with term-born controls. For this purpose, we measured D_{LCO} with its subcomponents twice in two EP cohorts with matched term-born controls and constructed longitudinal trajectories from 10 to 18 years and 18 to 25 years of age.

Methods

Study subjects and study design

Two area-based cohorts of subjects born at gestational age ⩽28 weeks or with birthweight ⩽1000 g in 1982-1985 (n=48) and 1991-1992 (n=35) were included. Subjects were examined in 2001-2002 and 2008-2009 at Haukeland University Hospital (Bergen, Norway). The temporally nearest term-born same-sex subject with birthweight 3-4 kg (approximately Norwegian 10-90th percentiles) was invited as a control. If that subject declined, the next term-born was approached and so on. There were no exclusion criteria except inability to perform lung function tests. Clinical data were accessed from patients' hospital charts. The cohorts are described in detail elsewhere [20], and their neonatal and background data are summarised in tables 1 and 2. Mild and moderate/severe BPD were defined as a requirement for supplemental oxygen ⩾28 postnatal days or at postmenstrual age ⩾36 weeks, respectively [21]. No subjects were examined within 2 weeks of a respiratory tract infection or an asthma exacerbation. Participants were asked to discontinue inhaled long-acting β_2-agonists and corticosteroids as well as oral leukotriene blockers 24 h before testing, to avoid inhaled short-acting β_2-agonists unless needed, and to refrain from smoking on the test day. Data on self-reported smoking have been verified in the 1982-1985 cohort by measuring urinary cotinine, with three positive tests in 57 self-declared nonsmokers [22]. The Regional Ethics Committee approved the study (REK-Vest 240.07). Informed written consent was obtained from participating subjects and/or parents.

Pulmonary function tests

The same experienced respiratory physiologist (O.D.R.) performed all tests on pulmonary function on both occasions, blinded to the results obtained in previous test sessions. Single-breath D_{LCO} was measured with a Vmax 22 (SensorMedics, Yorba Linda, CA, USA) in the sitting position wearing a nose clip, in accordance with European Respiratory Society (ERS) guidelines [23].

Single-breath method

The test gas contained a mixture of 0.3% carbon monoxide, 0.3% methane and 21% oxygen (80% oxygen in the hyperoxic test gas), balanced with nitrogen. A mid-expiratory sample of alveolar gas was collected and analysed. Alveolar volume (V_A) and transfer coefficient of the lung for carbon monoxide (K_{CO}) were recorded and D_{LCO} calculated. D_M and V_C were measured with a hyperoxic test gas (80% oxygen) and calculated according to the Roughton-Forster equation [10]. Test criteria were applied as recommended by the ERS Task Force [23]. Details regarding the single-breath D_{LCO} measurements have previously been described [11]. z-scores for V_A, K_{CO} and D_{LCO} were calculated using the Global Lung Function Initiative 2017 regression equations (updated version, October 2020) for the carbon monoxide transfer factor for Caucasians [24].

Statistical methods

Results are reported as counts with proportions and means with 95% confidence intervals or ranges, as appropriate. The number of patients each analysis is based on is reported separately due to some missing data, particularly at the second follow-up (figure 1).

TABLE 1 Neonatal characteristics of extremely preterm (EP) subjects (n=83)

	1991-1992 cohort		1982-1985 cohort	
	Mean (range) or n (%)	SD	Mean (range) or n (%)	SD
Birthweight, g				
All EP	933 (570-1400)	204	1012 (580-1480)	189
No/mild BPD	976 (620-1400)	195	1056 (580-1480)	191
Moderate/severe BPD	851 (570-1200)	203	892 (670-1080)	122
Gestational age, weeks				
All EP	27 (23-31)	2	27 (23-32)	1
No/mild BPD	27 (24-31)	2	27 (23-32)	2
Moderate/severe BPD	26 (23-28)	1	27 (26-30)	1
Postnatal time with oxygen, days				
All EP	57 (2-180)	48	48 (1-257)	39
No/mild BPD	31 (2-70)	23	33 (1-71)	18
Moderate/severe BPD	108 (61-180)	43	85 (44-257)	54
Time on ventilator, days				
All EP	8 (0-55)	12	11 (0-54)	12
No/mild BPD	4 (0-40)	9	7 (0-35)	8
Moderate/severe BPD	16 (2-55)	13	21 (1-54)	16
Antenatal steroids				
All EP	16 (46)		16 (33)	
No/mild BPD	11 (48)		10 (29)	
Moderate/severe BPD	5 (42)		6 (46)	
Surfactant				
All EP	17 (49)		0 (0)	
No/mild BPD	7 (30)		0 (0)	
Moderate/severe BPD	10 (83)		0 (0)	
Postnatal steroids				
All EP	10 (29)		4 (8)	
No/mild BPD	2 (9)		1 (3)	
Moderate/severe BPD	8 (67)		3 (23)	
Maternal smoking in pregnancy				
All EP	13 (37)		22 (48)	
No/mild BPD	10 (43)		17 (50)	
Moderate/severe BPD	3 (27)		5 (42)	

BPD: bronchopulmonary dysplasia (no/mild BPD: no need for oxygen supplementation at 36 weeks postmenstrual age; moderate/severe BPD: oxygen supplement at 36 weeks postmenstrual age). The number of subjects differed slightly between variables. In the 1991-1992 cohort: all EP n=34-35 subjects, no/mild BPD n=23 subjects and moderate/severe BPD n=11-12 subjects. In the 1982-1985 cohort: all EP n=46-48 subjects, no/mild BPD n=34-35 subjects and moderate/severe BPD n=12-13 subjects.

To estimate mean values and differences in mean values for the clinical variables z-D_{LCO}, D_{LCO} % pred, z-V_A, z-K_{CO}, K_{CO} % pred, D_M and V_C for the two groups at each time-point, we fitted linear mixed effects longitudinal models. The explanatory variables were cohort, age (categorical) and EP versus term-born (or grade of BPD severity in supplementary table B). To make the models maximally flexible, we included all interactions. Subjects were included as a random effect (as expected, there was no "EP-term-born pair" effect, so this was not included as a random effect). These models take the correlations between measurements at various follow-up times from the same subject into account, which makes it possible to also include subjects with incomplete follow-up data. This was done to reduce any bias caused by missing data and to increase the precision of the estimates [25]. Residual plots were examined and any errors in the original data corrected. To examine if development for EP subjects tracked development for term-born subjects, we fitted simplified models with parallel lines for the two groups (but possibly different slopes for the two cohorts) and compared these with the fully flexible models using likelihood ratio tests. To examine the effects of smoking, and if smoking impacted EP and term-born differently, we added smoking and the interaction EP versus term-born to the z-D_{LCO} model.

Associations between perinatal exposures and outcome were tested in a linear regression model with z-D_{LCO} at 18 years of age (in both cohorts) as response variable and the following as explanatory variables: maternal smoking, gestational age, antenatal steroids, surfactant, days on mechanical ventilation

TABLE 2 Background variables for extremely preterm (EP) subjects and term-born controls

	1991-1992 cohort		1982-1985 cohort	
	First follow-up	Second follow-up	First follow-up	Second follow-up
Age, years (mean±SD)	10.6±0.4	17.8±0.4	17.7±1.2	24.9±1.2
Subjects, n (% females)				
Term-born	35 (63)	28 (71)	46 (46)	40 (4)
EP	35 (63)	31 (58)	46 (46)	45 (42)
Height, cm				
Term-born				
Female	144 (141-147)	166 (163-168)	168 (165.4-171.0)	168 (165-171)
Male	145 (142-150)	178 (171-185)	177 (174-179)	177 (175-180)
EP				
Female	141 (137-145)	162 (159-166)	163 (162-166)	163 (162-165)
Male	139 (135-143)	174 (171-178)	175 (172-177)	176 (173-178)
Weight, kg				
Term-born				
Female	39 (36-41)	64 (59-69)	67 (60-73)	69 (61-77)
Male	38 (34-42)	75 (65-85)	68 (65-71)	76 (71-81)
EP				
Female	35 (30-41)	62 (51-72)	61 (53-68)	67 (57-76)
Male	35 (27-42)	73 (62-83)	66 (59-72)	80 (74-87)
Self-reported smoking				
Term-born	0 (0)	5 (18)	14 (30)	8 (21)
EP	0 (0)	1 (3)	15 (33)	17 (38)
Maternal smoking in pregnancy				
Term-born	9 (26)		10 (22)	
EP	13 (37)		22 (48)	
z-FEV_1				
Term-born	−0.09 (−0.4-0.2)	−0.06 (−0.5-0.3)	0.3 (−0.4-0.6)	0.05 (−0.3-0.4)
EP	−0.9 (−1.2- −0.6)	−0.8 (−1.1- −0.5)	−1.05 (−1.6- −0.5)	−1.0 (−1.5- −0.5)
z-FVC				
Term-born	−0.1 (−0.4-0.2)	−0.08 (−0.4-0.2)	−0.05 (−0.4-0.3)	0.09 (−0.3-0.4)
EP	−0.6 (−0.9- −0.3)	−0.3 (−0.6-0.03)	−0.9 (−1.5- −0.4)	−0.5 (−1.0-0.04)
z-$FEF_{25-75\%}$				
Term-born	−0.2 (−0.6-0.2)	−0.2 (−0.6-0.3)	0.5 (0.1-0.8)	−0.004 (−0.3-0.3)
EP	−1.1 (−1.5- −0.7)	−0.9 (−1.3- −0.5)	0.8 (−1.2- −0.5)	−1.2 (−1.5- −0.8)

Data are presented as group means (95% CI) or n (%), unless otherwise stated. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25-75\%}$: forced expiratory flow at 25-75% of FVC.

(with values >30 days set to 30 days), days on oxygen supplementation (with values >100 days set to 100 days) and cohort.

For the background variables (tables 1 and 2), differences between groups were assessed with Welch's t-test for continuous variables and Pearson's Chi-squared test for categorical variables.

The original project was designed in 2001 to address a series of outcomes and the sample size was calculated to detect a clinically relevant decrease in the EP groups for the main outcome measure for the overall study, which was forced expiratory volume in 1 s (FEV_1) [22].

The data were analysed with SPSS version 25 (IBM, Armonk, NY, USA) and R version 4.0.2 [26]. The mixed effects models were fitted with the R package "lme4" version 1.1-23 [27]. p-values $\leqslant 0.05$ are characterised as statistically significant.

Results

Subjects

A total of 130 preterms were admitted to the neonatal intensive care unit (NICU) in the two inclusion periods. Neonatal mortality was 39% and 27% in 1982-1985 and 1991-1992, respectively. Altogether in

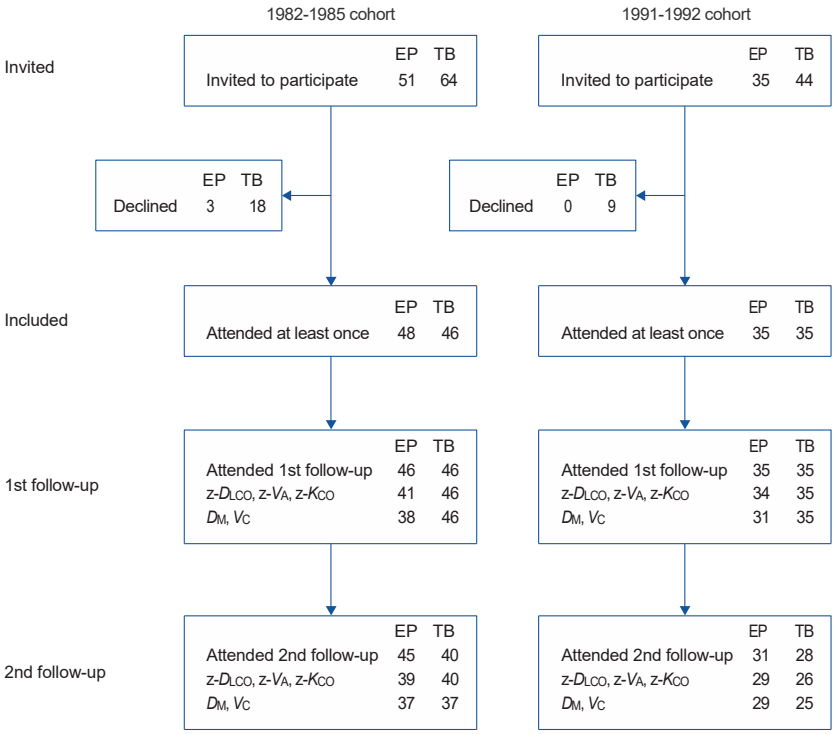


FIGURE 1 Recruitment process of the study (n=164). Recruitment of the extremely preterm (EP) cohorts and their term-born (TB) age- and sex-matched control subjects. Two subjects in the 1982-1985 cohort participated in the second follow-up in 2008-2009 but not in the first follow-up in 2001-2002. D_{LCO}: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume; K_{CO}: transfer coefficient of the lung for carbon monoxide; D_M: alveolar-capillary membrane conductance; V_C: pulmonary capillary blood volume.

both EP cohorts, 86 subjects survived, 81 attended the first follow-up, 74 attended both follow-ups and 83 attended at least one follow-up.

Subject demographics are summarised in table 1. Mean gestational age was similar in both cohorts. The younger cohort had fewer days on a ventilator, and higher use of antenatal and postnatal steroids. No subjects in the 1982-1985 cohort received surfactant, contrasting with almost half of the EP subjects in the 1991-1992 cohort (table 1).

There were no differences between the EP and term-born subjects regarding weight (table 2). Regarding height, EP females in the 1982-1985 cohort were significantly shorter at both examinations (both p=0.006), as were EP males in the 1991-1992 cohort at the first (p=0.01) but not the second examination (p=0.29).

Most participants were able to perform D_{LCO} measurements (figure 1). Success rates at first follow-up (ages 10 and 18 years) were 97% and 89% for EP subjects and 100% for term-born subjects (both cohorts). Corresponding numbers at second follow-up (ages 18 and 25 years) were 94% and 87% for EP subjects and 93% and 100% for term-born subjects. Some of those who struggled with performing satisfactory measurements at 21% oxygen did not perform measurements at 80% oxygen, and thus D_M and V_C measurements were obtained for fewer subjects (figure 1).

D_{LCO}, V_A and K_{CO}

Raw data (for D_{LCO} and K_{CO}) are presented in supplementary table A, whereas z-scores are used in figure 2 and table 3. Table 3 also includes percentage predicted values. z-D_{LCO} and z-K_{CO} were lower in EP

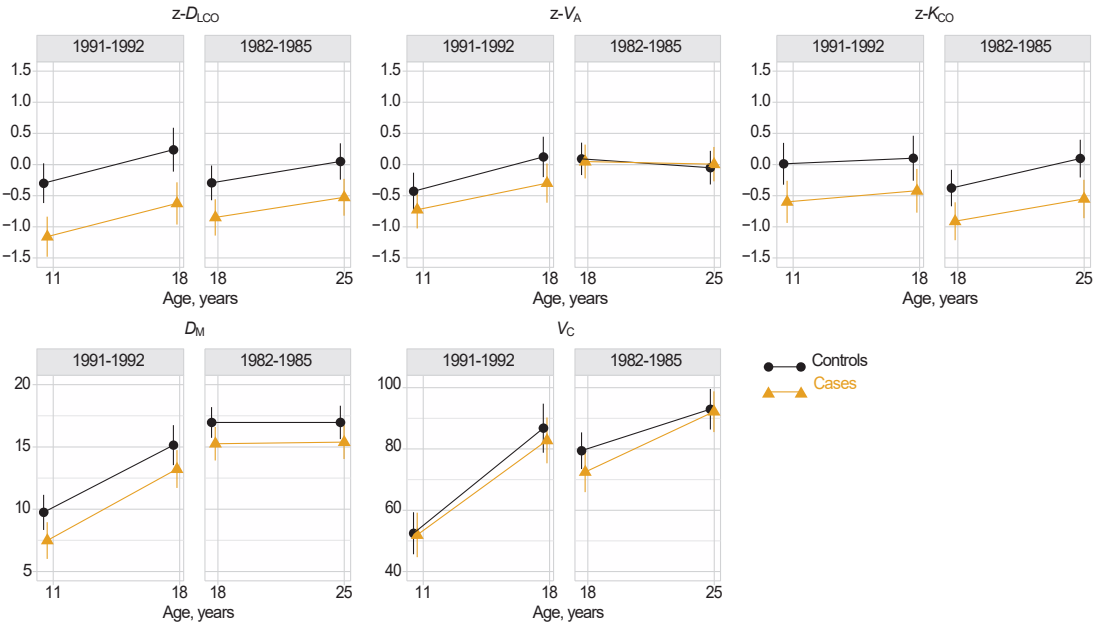


FIGURE 2 Mean lung diffusing capacity from approximately 10 to 25 years of age for extremely preterm subjects compared with term-born controls (n=160[#]). Data are presented as estimated group means (95% CI) from longitudinal mixed effects models. The points/lines for the two groups have been slightly adjusted horizontally to avoid overlapping. The values for diffusing capacity of the lung for carbon monoxide (D_{LCO}), alveolar volume (V_A) and transfer coefficient of the lung for carbon monoxide (K_{CO}) are reported as z-scores, while values for alveolar-capillary membrane conductance (D_M; mmol·min^{-1}·kPa^{-1}) and pulmonary capillary blood volume (V_C; mL) are absolute numbers.[#]: number of subjects included in at least one regression model (numbers of cases and controls for each variable and at each time-point are shown in figure 1).

compared with term-born subjects in both cohorts and at both examinations, whereas z-V_A was similar. Data for EP subjects split by neonatal BPD is presented in supplementary table B. Within the EP cohorts, BPD did not influence z-D_{LCO}, z-V_A and z-K_{CO} (p-values of 0.14, 0.45 and 0.15, respectively).

Smokers had on average 0.6 lower z-D_{LCO} values (95% CI 0.2-1.0; p=0.002). The effect did not differ between the EP and term-born subjects (p=0.31 for the interaction).

There were no associations between the addressed perinatal variables or cohort versus z-D_{LCO} at 18 years of age, with p-values of 0.28, 0.12, 0.21, 0.93, 0.90, 0.66 and 0.74 for maternal smoking, gestational age, antenatal steroids, surfactant, days on mechanical ventilation, days on oxygen supplementation and cohort, respectively.

D_M and V_C

D_M was numerically lower in the EP compared with term-born cohorts, statistically significantly so only in the 1991-92 cohort at 10 years of age. V_C did not differ between the EP and term-born cohorts at any of the measurements.

Development over time

For both EP cohorts, z-D_{LCO}, z-K_{CO}, z-V_A, D_M and V_C developed in parallel to their respective term-born control cohorts over the age span covered by the study, i.e. from 18 to 25 years of age in the 1982-1985 cohort and from 10 to 18 years of age in the 1991-1992 cohort.

The p-values for overall tests for a lack of parallelism between the EP and term-born cohorts from each of the two decades were 0.99, 0.65, 0.71, 0.94 and 0.44 for z-D_{LCO}, z-V_A, z-K_{CO}, D_M and V_C, respectively. This indicates that development between the two examinations did not differ between the EP and term-born groups for any of the measured variables.

TABLE 3 Lung diffusing capacity data from 10 to 25 years of age for extremely preterm (EP) subjects compared with term-born controls (n=160[#])

	1991-1992 cohort				1982-1985 cohort			
	First follow-up	p-value	Second follow-up	p-value	First follow-up	p-value	Second follow-up	p-value
Age, years (mean±SD)	10.6±0.4		17.8±0.4		17.7±1.2		24.9±1.2	
z-D_{LCO}								
Term-born	−0.3 (−0.6-0.0)		0.2 (−0.1-0.6)		−0.3 (−0.6- −0.0)		0.0 (−0.2-0.3)	
EP	−1.2 (−1.5- −0.8)		−0.6 (−1.0- −0.3)		−0.8 (−1.1- −0.6)		−0.5 (−0.8- −0.2)	
Difference	0.9 (0.4-1.3)	<0.001	0.9 (0.4-1.4)	<0.001	0.6 (0.2-1.0)	0.007	0.6 (0.2-1.0)	0.007
D_{LCO}, % pred								
Term-born	95.7 (91.3-100.1)		104.0 (99.1-108.9)		96.6 (92.8-100.4)		101.3 (97.3-105.3)	
EP	83.1 (78.6-87.5)		92.1 (87.5-96.8)		89.1 (85.1-93.2)		93.7 (89.6-97.8)	
Difference	12.6 (6.4-18.9)	<0.001	11.9 (5.1-18.6)	<0.001	7.4 (1.9-13)	0.009	7.6 (1.9-13.4)	0.009
z-V_A								
Term-born	−0.4 (−0.7- −0.1)		0.1 (−0.2-0.4)		0.1 (−0.2-0.4)		−0.1 (−0.3-0.2)	
EP	−0.7 (−1.0- −0.4)		−0.3 (−0.6-0.0)		0.0 (−0.2-0.3)		0.0 (−0.3-0.3)	
Difference	0.3 (−0.1-0.7)	0.17	0.4 (−0.0-0.9)	0.07	0.0 (−0.3-0.4)	0.82	−0.1 (−0.4-0.3)	0.77
z-K_{CO}								
Term-born	0.0 (−0.3-0.3)		0.1 (−0.3-0.5)		−0.4 (−0.7- −0.1)		0.1 (−0.2-0.4)	
EP	−0.6 (−0.9- −0.3)		−0.4 (−0.8- −0.1)		−0.9 (−1.2- −0.6)		−0.6 (−0.9- −0.2)	
Difference	0.6 (0.1-1.1)	0.01	0.5 (0.0-1.0)	0.04	0.5 (0.1-1.0)	0.01	0.6 (0.2-1.1)	0.003
K_{CO}, % pred								
Term-born	100.5 (95.9-105.1)		101.5 (96.5-106.4)		95.5 (91.5-99.5)		101.9 (97.8-106.1)	
EP	90.5 (85.9-95.1)		94.9 (90.1-99.7)		88.6 (84.4-92.7)		93.7 (89.5-97.9)	
Difference	10.0 (3.5-16.4)	0.003	6.6 (−0.3-13.5)	0.06	6.9 (1.2-12.7)	0.02	8.2 (2.3-14.1)	0.006
D_M								
Term-born	9.7 (8.3-11.2)		15.1 (13.5-16.7)		17.0 (15.7-18.2)		17.0 (15.6-18.3)	
EP	7.5 (6.0-9.0)		13.2 (11.7-14.7)		15.3 (13.9-16.6)		15.4 (14.0-16.7)	
Difference	2.3 (0.2-4.3)	0.03	1.9 (−0.3-4.1)	0.09	1.7 (−0.1-3.5)	0.07	1.6 (−0.3-3.5)	0.10
V_C								
Term-born	52.5 (45.6-59.4)		86.8 (78.8-94.8)		79.4 (73.4-85.4)		93.0 (86.4-99.6)	
EP	51.9 (44.7-59.2)		82.8 (75.3-90.3)		72.5 (65.9-79.0)		92.2 (85.5-98.8)	
Difference	0.5 (−9.4-10.5)	0.91	4.0 (−7.0-14.9)	0.47	7.0 (−1.9-15.8)	0.12	0.8 (−8.6-10.2)	0.87

Data are presented as group means (95% CI) from longitudinal mixed effects models, unless otherwise stated. D_{LCO}: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume; K_{CO}: transfer coefficient of the lung for carbon monoxide; D_M: alveolar-capillary membrane conductance; V_C: pulmonary capillary blood volume. The values for D_{LCO}, V_A and K_{CO} are reported as z-scores and percentage predicted values (D_{LCO} and K_{CO}), while values for D_M (mmol·min^{-1}·kPa^{-1}) and V_C (mL) are absolute numbers.[#]: number of subjects included in at least one regression model (numbers of cases and controls for each variable and at each time-point are shown in figure 1).

Discussion

This is the first controlled population-based study describing longitudinal development of lung diffusing capacity after EP birth from mid-childhood to adulthood. We found that D_{LCO} and K_{CO} were persistently reduced in EP subjects, and that development tracked below but in parallel to term-born subjects over the study period, with no signs of pubertal catch-up growth nor any signs of decline at 25 years age. Split by membrane and blood volume components, findings were less clear; however, the membrane diffusion component seemed most affected.

Gas exchange takes place in the acini, where air and blood are in proximity, with an ultrathin alveolar-capillary membrane separating the compartments. The diffusing capacity of the lungs is structurally limited by the magnitude of the alveolar surface area, the thickness of the blood-gas barrier and the pulmonary capillary blood volume. Formation of the alveoli is the final stage of lung development and much of this process takes place after birth also in term-born individuals [28]. Nevertheless, postnatal development builds on premises established during the last trimester, which is a period EP subjects spend in the NICU. New alveoli form by alveolar ducts dividing into alveolar sacs by septation and the pulmonary capillary bed expands in parallel via angiogenesis, gradually increasing the area available for gas exchange [17]. This is a continuous process that commences in the last trimester and continues for years after birth. EP birth, with accompanying dramatic events and lifesaving respiratory interventions, radically changes the premises under which this developmental programme must take place. Autopsy studies of children who died from BPD have shown impaired acinar development [6, 7, 29], but we have little knowledge of structural features in survivors and future prospects for growth or repair after the neonatal period are unknown. Judged by aerosol-derived airway morphometry studies, the size of a child's alveolus expands

into adulthood, accounting for increased lung volume with age and height [30]. On the other hand, studies applying stereological approaches indicate that the alveolar number closely relates to total lung volume, with a constant alveolar size over a range of volumes, suggesting that the number of alveoli must increase during growth [31]. Thus, the surface area available for gas exchange is much larger in an adult compared with a child. This relatively simple line of reasoning was recently confirmed by MRI studies showing continued alveolarisation to adolescence and catch-up growth in EP children [8]. These studies provide optimism that repair mechanisms might come into play as preterm-born children grow and mature. However, judged by the development of the pulmonary capacity for carbon monoxide transfer of the two EP cohorts in our study, a corresponding functional catch-up is difficult to detect; the EP cohorts had consistently reduced gas exchange capacity that tracked from 10 to 25 years of age.

At quiet breathing, both volume and effective surface area of the capillary bed change with changes of the blood flow that reflect the stroke volume [32]. The blood stays very briefly in the pulmonary capillaries, but still, venous blood entering the lung capillaries equilibrates completely with alveolar air in a highly efficient process requiring ~0.3 s. Healthy individuals have large ventilatory reserves and deficits in gas diffusing capacity are therefore well tolerated at rest. During exercise, the transit time through the lung capillaries is shortened, challenging gas exchange capacity in patients with low D_{LCO}, with 50% predicted suggested as a threshold before symptoms occur [33-35]. In EP subjects, deficits in D_{LCO} raw data are generally ~10% [13, 16], which was also found in the present study (supplementary table A). We have previously shown that compared with controls, these same EP subjects have close-to-normal peak exercise capacity [36], findings replicated also by others [13, 37, 38]. Taken together, close-to-normal gas diffusing and exercise capacities challenge the notion that EP birth leads to severe persistent acinar impairment, since one would expect more austere physiological findings if this was the case. Airway obstruction can lead to a higher D_{LCO} [39] and we know that EP subjects (including our cohorts) have persistent airway obstruction, particularly those who had neonatal BPD [40, 41]. Thus, bronchial obstruction might mask or counteract deficits of D_{LCO} and therefore explain a surprising finding in our dataset, i.e. that EP subjects with BPD tended to have a higher z-D_{LCO}, although not significant (supplementary table B).

Impaired alveolar development could theoretically hamper lung diffusing capacity through reduced area and/or a thickening or impairment of the alveolar-capillary membrane, or by impaired vascular components. We found that the membrane component of D_{LCO} was numerically reduced at both measurement time-points in both EP cohorts, although significantly so only at the first examination in the youngest cohort. Data for V_C did not exhibit corresponding deficits and increased over time in both cohorts, also from 18 to 25 years of age, indicating similar growth and development in the EP and term-born groups, presumably in parallel to the increases in body size. Reduced D_{LCO} in the EP groups must reflect comparable reductions in its subcomponents D_M and/or V_C. Given the data of our study, it is enticing to conclude that reduced D_{LCO} after preterm birth is more related to impairments of membrane diffusion than the vascular components of acinar development. Future studies preferably including more participants may disentangle the underlying mechanisms of impaired D_{LCO} following EP birth.

Disruption of alveolar growth associated with EP birth may be linked to early-onset chronic obstructive pulmonary disease (COPD) in adult life [42]. In clinical practice, D_{LCO} is used to assess severity and prognosis of COPD, as spirometry alone poorly reflects the disability in these patients. Reduced D_{LCO} is a prognostic marker independent of forced spirometry in COPD patients [43] and is associated with increased morbidity across multiple domains [43]. Moreover, lung diffusing capacity has been shown to be a significant predictor of the all-cause mortality rate within a general population, independent of standard spirometry measures and even in the absence of apparent clinical respiratory disease [44]. There is ample data to argue that airway obstruction tracks at a reduced level from EP birth via early childhood to adulthood and that few of these individuals reach their expected peak FEV_1 [41, 45]. Our study indicates a similar tracking also for D_{LCO}, a scenario suggesting that D_{LCO} should be included in follow-up programmes after EP birth.

Strengths and limitations

The major strengths of the study were the population-based, longitudinal and controlled design, the long follow-up period, and the high rate of attendance at both follow-up assessments of both EP and term-born participants. A strict algorithm for recruitment of control subjects minimised the risk of selection bias in this group. Development during the age span covered by the study was described by examining two birth cohorts that overlapped in age but were born during two different NICU eras. This was not an ideal approach to address longitudinal development over the full recruitment period, but we consider it adequate to compare the trajectories for the EP and term-born groups. We cannot comment on a potential for

early-onset age-related decline of D_{LCO}, as studies of the general population have shown that the decline starts to accelerate later than by the age of 25 years [46].

Preterm infants were exposed to very different treatment algorithms and techniques in the 1980s and the 1990s, reflected in this study by, for example, a higher rate of subjects treated with antenatal steroids and surfactant, and also by a higher rate of survival in the younger cohort. Caution is therefore warranted for direct comparisons between the cohorts. However, this line of thinking can be turned the other way around, i.e. one may argue that similar findings in two birth cohorts born 9 years apart and treated so very differently strengthen the notion that parameters of lung diffusion are in fact tracking from childhood to adulthood. The continuous development of NICU treatment during the last decades challenges the generalisability of these findings to today's NICU dwellers, as their outcomes may differ. Numerically, our data from all the various measurement time-points were in line with most previous reports on lung diffusing capacity [18, 47]. As observed also by others, we found no clear associations in our dataset between neonatal BPD and subsequent lung diffusing capacity [13, 15, 16]. Recent studies of other indices of lung function in groups and cohorts born EP in the modern era of neonatology also suggest weaker associations with BPD [48]. Thus, we should perhaps contemplate revising the use of this neonatal diagnosis to predict and label subsequent lung function in EP adults.

Asthma therapy was stopped ~24 h prior to testing. This time frame did not allow full washout of inhaled corticosteroids. D_{LCO} is linked to the ventilation/perfusion ratio, which may be affected by bronchial obstruction. Increased airway conductance and accessible alveolar volume caused by sustained effects from asthma therapy could possibly influence the findings in the few participants with asthma. The available data in this area is scarce [49] and the 2017 ERS/American Thoracic Society standards for single-breath carbon monoxide uptake in the lung do not provide specific advice regarding discontinuation of asthma medications [9].

Inclusion to this study was based on both gestational age and birthweight criteria, preventing generalisation of the results to all EP cohorts, as some dysmature infants were included based on the birthweight criteria alone. Potential relationships between perinatal characteristics and subsequent measures of pulmonary gas transfer should be addressed in future, larger studies.

Conclusions

EP subjects had impaired lung diffusing capacity, with membrane diffusion seemingly more implicated than the capillary blood volume component. The deficits tracked from mid-childhood to adulthood, below but in parallel to matched term-born control cohorts. Preterm birth represents a significant perturbation to lung development in the short term but also long term. A lifelong obligation for proper follow-up, treatment and guidance falls upon the health profession that once made survival of these young individuals possible.

Conflict of interest: None declared.

References

1 Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012; 379: 2162-2172.
2 Stensvold HJ, Klingenberg C, Stoen R, et al. Neonatal morbidity and 1-year survival of extremely preterm infants. Pediatrics 2017; 139: e2016182.
3 Smith LJ, McKay KO, van Asperen PP, et al. Normal development of the lung and premature birth. Paediatr Respir Rev 2010; 11: 135-142.
4 Baker CD, Alvira CM. Disrupted lung development and bronchopulmonary dysplasia: opportunities for lung repair and regeneration. Curr Opin Pediatr 2014; 26: 306-314.
5 Thebaud B, Goss KN, Laughon M, et al. Bronchopulmonary dysplasia. Nat Rev Dis Primers 2019; 5: 78.
6 Thibeault DW, Mabry SM, Norberg M, et al. Lung microvascular adaptation in infants with chronic lung disease. Biol Neonate 2004; 85: 273-282.
7 Husain AN, Siddiqui NH, Stocker JT. Pathology of arrested acinar development in postsurfactant bronchopulmonary dysplasia. Hum Pathol 1998; 29: 710-717.
8 Narayanan M, Beardsmore CS, Owers-Bradley J, et al. Catch-up alveolarization in ex-preterm children: evidence from ^{3}He magnetic resonance. Am J Respir Crit Care Med 2013; 187: 1104-1109. 9 Graham BL, Brusasco V, Burgos F, et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur Respir J 2017; 49: 1600016.

10 Roughton FJ, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 1957; 11: 290-302.
11 Satrell E, Roksund O, Thorsen E, et al. Pulmonary gas transfer in children and adolescents born extremely preterm. Eur Respir J 2013; 42: 1536-1544.
12 Lum S, Kirkby J, Welsh L, et al. Nature and severity of lung function abnormalities in extremely preterm children at 11 years of age. Eur Respir J 2011; 37: 1199-1207.
13 Vrijlandt EJ, Gerritsen J, Boezen HM, et al. Lung function and exercise capacity in young adults born prematurely. Am J Respir Crit Care Med 2006; 173: 890-896.
14 Sorensen JK, Buchvald F, Berg AK, et al. Ventilation inhomogeneity and NO and CO diffusing capacity in ex-premature school children. Respir Med 2018; 140: 94-100.
15 Hakulinen AL, Jarvenpaa AL, Turpeinen M, et al. Diffusing capacity of the lung in school-aged children born very preterm, with and without bronchopulmonary dysplasia. Pediatr Pulmonol 1996; 21: 353-360.
16 Kaplan E, Bar-Yishay E, Prais D, et al. Encouraging pulmonary outcome for surviving, neurologically intact, extremely premature infants in the postsurfactant era. Chest 2012; 142: 725-733.
17 Hislop AA. Airway and blood vessel interaction during lung development. J Anat 2002; 201: 325-334. Chang
18 DV, Assaf SJ, Tiller CJ, et al. Membrane and capillary components of lung diffusion in infants with bronchopulmonary dysplasia. Am J Respir Crit Care Med 2016; 193: 767-771.
19 Drummond D, Hadchouel A, Le Bourgeois M, et al. Decreased pulmonary capillary volume in adolescents born very preterm. Acta Paediatr 2020; 109: 621-622.
20 Halvorsen T, Skadberg BT, Eide GE, et al. Assessment of lung volumes in children and adolescents: comparison of two plethysmographic techniques. Clin Physiol Funct Imaging 2005; 25: 62-68.
21 Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001; 163: 1723-1729.
22 Vollsaeter M, Clemm HH, Satrell E, et al. Adult respiratory outcomes of extreme preterm birth. A regional cohort study. Ann Am Thorac Soc 2015; 12: 313-322.
23 Macintyre N, Crapo RO, Viegi G, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J 2005; 26: 720-735.
24 Stanojevic S, Graham BL, Cooper BG, et al. Official ERS technical standards: global lung function initiative reference values for the carbon monoxide transfer factor for Caucasians. Eur Respir J 2017; 50: 1700010.
25 Mallinckrodt CH, Clark WS, David SR. Accounting for dropout bias using mixed-effects models. J Biopharm Stat 2001; 11: 9-21.
26 R Core Team. R: A Language and Environment for Statistical Computing. Vienna, R Foundation for Statistical Computing, 2020.
27 Bates DMM, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw 2015; 67: 1-48.
28 Burri PH. Structural aspects of postnatal lung development - alveolar formation and growth. Biol Neonate 2006; 89: 313-322.
29 Hislop AA, Wigglesworth JS, Desai R, et al. The effects of preterm delivery and mechanical ventilation on human lung growth. Early Hum Dev 1987; 15: 147-164.
30 Zeman KL, Bennett WD. Growth of the small airways and alveoli from childhood to the adult lung measured by aerosol-derived airway morphometry. J Appl Physiol 2006; 100: 965-971.
31 Ochs M, Nyengaard JR, Jung A, et al. The number of alveoli in the human lung. Am J Respir Crit Care Med 2004; 169: 120-124.
32 Johnson RL Jr, Spicer WS, Bishop JM, et al. Pulmonary capillary blood volume, flow and diffusing capacity during exercise. J Appl Physiol 1960; 15: 893-902.
33 Sue DY, Oren A, Hansen JE, et al. Diffusing capacity for carbon monoxide as a predictor of gas exchange during exercise. N Engl J Med 1987; 316: 1301-1306.
34 Hadeli KO, Siegel EM, Sherrill DL, et al. Predictors of oxygen desaturation during submaximal exercise in 8,000 patients. Chest 2001; 120: 88-92.
35 Bedell GN, Adams RW. Pulmonary diffusing capacity during rest and exercise. A study of normal persons and persons with atrial septal defect, pregnancy, and pulmonary disease. J Clin Invest 1962; 41: 1908-1914.
36 Clemm HH, Vollsaeter M, Roksund OD, et al. Exercise capacity after extremely preterm birth: development from adolescence to adulthood. Ann Am Thorac Soc 2014; 11: 537-545.
37 Kilbride HW, Gelatt MC, Sabath RJ. Pulmonary function and exercise capacity for ELBW survivors in preadolescence: effect of neonatal chronic lung disease. J Pediatr 2003; 143: 488-493.
38 Burns YR, Danks M, O'Callaghan MJ, et al. Motor coordination difficulties and physical fitness of extremely-low-birthweight children. Dev Med Child Neurol 2009; 51: 136-142.
39 Collard P, Njinou B, Nejadnik B, et al. Single breath diffusing capacity for carbon monoxide in stable asthma. Chest 1994; 105: 1426-1429.
40 Doyle LW, Andersson S, Bush A, et al. Expiratory airflow in late adolescence and early adulthood in individuals born very preterm or with very low birthweight compared with controls born at term or with normal birthweight: a meta-analysis of individual participant data. Lancet Respir Med 2019; 7: 677-686.

41 Vollsaeter M, Roksund OD, Eide GE, et al. Lung function after preterm birth: development from mid-childhood to adulthood. Thorax 2013; 68: 767-776.
42 McGrath-Morrow SA, Collaco JM. Bronchopulmonary dysplasia: what are its links to COPD? Ther Adv Respir Dis 2019; 13: 1753466619892492.
43 Balasubramanian A, MacIntyre NR, Henderson RJ, et al. Diffusing capacity of carbon monoxide in assessment of COPD. Chest 2019; 156: 1111-1119.
44 Neas LM, Schwartz J. Pulmonary function levels as predictors of mortality in a national sample of US adults. Am J Epidemiol 1998; 147: 1011-1018.
45 Moschino L, Stocchero M, Filippone M, et al. Longitudinal assessment of lung function in survivors of bronchopulmonary dysplasia from birth to adulthood. The Padova BPD study. Am J Respir Crit Care Med 2018; 198: 134-137.
46 Stam H, Hrachovina V, Stijnen T, et al. Diffusing capacity dependent on lung volume and age in normal subjects. J Appl Physiol 1994; 76: 2356-2363.
47 Arigliani M, Valentini E, Stocco C, et al. Regional ventilation inhomogeneity in survivors of extremely preterm birth. Pediatr Pulmonol 2020; 55: 1366-1374.
48 Hjalmarson O, Brynjarsson H, Nilsson S, et al. Persisting hypoxaemia is an insufficient measure of adverse lung function in very immature infants. Arch Dis Child Fetal Neonatal Ed 2014; 99: F257-F262.
49 Yang J, Stanton J, Wang L, et al. Effect of salbutamol on the measurement of single-breath diffusing capacity. Respirology 2013; 18: 1223-1229.

Supplemental Table A. *Raw data for lung diffusing capacity from 10 to 25 years of age for extreme preterm subjects compared to term-born control subjects (n = 160*).*

	1991-1992 cohort								1982-1985 cohort							
Examination	**First follow-up**				**Second follow-up**				**First follow-up**				**Second follow-up**			
Age, mean (SD)	10.6 (0.4) years				17.8 (0.4) years				17.7 (1.2) years				24.9 (1.2) years			
	Mean	95% CI			Mean	95% CI			Mean	95% CI			Mean	95% CI		
DL_{CO} ($mmol \cdot min^{-1} \cdot kPa^{-1}$)																
Term-born	5.4	5.2	to	5.6	8.7	7.8	to	9.6	8.9	8.3	to	9.5	9.6	8.9	to	10.4
EP-born	4.4	4.1	to	4.7	7.9	7.2	to	8.6	8.0	7.4	to	8.5	8.7	8.1	to	9.4
V_A (liter)																
Term-born	3.0	2.9	to	3.1	5.2	4.8	to	5.6	5.6	5.3	to	5.9	5.9	5.5	to	6.3
EP-born	2.7	2.5	to	2.8	5.0	4.6	to	5.3	5.3	5.0	to	5.6	5.7	5.3	to	6.0
K_{CO} ($mmol \cdot min^{-1} \cdot kPa^{-1} \cdot L^{-1}$)																
Term-born	1.8	1.8	to	1.9	1.7	1.6	to	1.8	1.6	1.5	to	1.7	1.6	1.6	to	1.7
EP-born	1.7	1.6	to	1.7	1.6	1.5	to	1.7	1.5	1.4	to	1.6	1.5	1.5	to	1.6
D_M ($mmol \cdot min^{-1} \cdot kPa^{-1}$)																
Term-born	9.7	9.0	to	10.5	15.1	13.6	to	16.6	17.0	15.6	to	18.4	17.0	15.6	to	18.5
EP-born	7.4	6.5	to	8.3	13.2	11.5	to	14.8	15.0	13.4	to	16.6	15.3	13.4	to	17.2
V_C (mL)																
Term-born	52.5	48.2	to	56.8	86.9	73.5	to	100.2	79.4	73.8	to	85.1	92.9	85.2	to	100.6
EP-born	51.3	43.5	to	59.0	52.6	74.8	to	90.4	72.6	68.0	to	77.1	91.8	84.5	to	99.2

Abbreviations: SD: standard deviation; CI: confidence interval; DL_{CO}: Diffusing capacity of the lung for carbon monoxide; V_A: Alveolar volume; K_{CO}: Transfer coefficient of the lung for carbon monoxide; D_M: Alveolar-capillary membrane conductance; V_C: Pulmonary-capillary blood volume.

The numbers are estimated group means with 95% confidence interval. The values are reported as absolute numbers.
* The number of subjects included in at least one regression model. The number of cases and controls for each variable and at each time point is shown in Figure 1 in the main paper.

***Supplemental Table B**- Lung Diffusing capacity in two cohorts of extremely preterm and term-born subjects born 1991-1992 and 1982-1985, stratified by grade of BPD severity (n = 160*).*

	1991-1992 cohort						1982-1985 cohort					
Examination	**First follow-up**			**Second follow-up**			**First follow-up**			**Second follow-up**		
Age, mean (SD)	10.6 (0.4) years			17.8 (0.4) years			17.7 (1.2) years			24.9 (1.2) years		
	Mean	95% CI		Mean	95% CI		Mean	95% CI		Mean	95% CI	
Z-DLco												
Term-born	−0.3	−0.6 to	0.0	0.2	−0.1 to	0.6	−0.3	−0.6 to	−0.0	0.0	−0.2 to	0.3
EP non/mild BPD	−1.4	−1.8 to	−1.0	−0.7	−1.1 to	−0.3	−0.8	−1.2 to	−0.5	−0.6	−1.0 to	−0.3
EP mod/sev BPD	−0.7	−1.2 to	−0.2	−0.4	−1.0 to	0.1	−0.9	−1.4 to	−0.3	−0.2	−0.8 to	0.4
***Z*-V_A**												
Term-born	−0.4	−0.7 to	−0.1	0.1	−0.2 to	0.4	0.1	−0.2 to	0.4	−0.0	−0.3 to	0.2
EP non/mild BPD	−0.8	−1.2 to	−0.4	−0.5	−0.9 to	−0.1	0.1	−0.2 to	0.4	0.0	−0.3 to	0.3
EP mod/sev BPD	−0.6	−1.1 to	−0.1	0.1	−0.5 to	0.6	−0.1	−0.6 to	0.4	−0.0	−0.5 to	0.5
***Z*-K_{CO}**												
Term-born	0.0	−0.3 to	0.3	0.1	−0.3 to	0.5	−0.4	−0.7 to	−0.1	0.1	−0.2 to	0.4
EP non/mild BPD	−0.8	−1.2 to	−0.3	−0.4	−0.8 to	0.1	−1.0	−1.3 to	−0.6	−0.6	−1.0 to	−0.3
EP mod/sev BPD	−0.3	−0.9 to	0.3	−0.5	−1.1 to	0.1	−0.8	−1.4 to	−0.2	−0.3	−0.9 to	0.3
D_M												
Term-born	9.7	8.3 to	11.2	15.1	13.5 to	16.7	17.0	15.7 to	18.2	17.0	15.6 to	18.3
EP non/mild BPD	7.7	5.8 to	9.5	12.4	10.5 to	14.3	15.5	13.9 to	17.1	15.5	13.9 to	17.1
EP mod/sev BPD	7.2	4.7 to	9.7	14.8	12.2 to	17.4	14.7	12.2 to	17.2	15.2	12.6 to	17.8
V_C												
Term-born	52.5	45.6 to	59.3	86.8	78.8 to	94.8	79.4	73.5 to	85.4	93.0	86.4 to	99.6
EP non/mild BPD	48.5	39.5 to	57.5	78.8	69.6 to	88.0	71.2	63.4 to	79.0	90.1	82.4 to	97.9
EP mod/sev BPD	58.2	46.0 to	70.3	90.4	77.7 to	103.1	75.6	63.4 to	87.8	97.5	84.7 to	110.2

Abbreviations: SD: standard deviation; CI: confidence interval; DL_{CO}: Diffusing capacity of the lung for carbon monoxide; V_A: Alveolar volume; K_{CO}: Transfer coefficient of the lung for carbon monoxide; D_M: Alveolar-capillary membrane conductance; V_C: Pulmonary-capillary blood volume.

The numbers are estimated group means with 95% confidence interval from longitudinal mixed-effects models. The values for DL_{CO}, V_A, and K_{CO} are reported as Z-scores, while values for D_M and V_C are absolute numbers. D_M= mmol/min/kPa, V_C= ml.

* The number of subjects included in at least one regression model. The number of cases and controls for each variable and at each time point is shown in Figure 1 in the main paper.

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

uib.no

ISBN: 9788230869581 (print)
9788230862667 (PDF)