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PULMONARY DISEASE IN INFANCY, PERINATAL INFLAMMATORY RISK FACTORS AND PROPHYLAXIS

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Life is not measured by the number of breaths you take, but by every moment that takes your breath away……. (anonymous author).

To Mats, Mikaela & Ludvig

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ABSTRACT

Objective

In the last decades the survival rate of preterm infants has increased substantially.[1-3] Despite improved intensive neonatal care the incidence of chronic lung disease in infants born preterm has not changed.[4-6] The overall aim of this thesis was to investigate pulmonary disease in infancy, perinatal inflammatory risk factors and prophylaxis, with specific emphasis on the development of bronchopulmonary dysplasia and the effect of antenatal corticosteroids.

Methods

This thesis was built on four observational population based studies. Studies I- III were cohort studies. Study IV was a case-control study. Studies I and II included infants born from 1976 through 1997 in Sweden and investigated the effect of antenatal corticosteroids exposure before gestational week 34. All infants in study I were born before gestational week 34, whereas infants in study II were born from gestational week 34 or later. Study III included infants born before gestational week 37, from 1988 through 2009 in Sweden and explored prenatal inflammatory risk factors for bronchopulmonary dysplasia. Study IV included infants born before gestational week 33, from 2005 through 2010 in Sweden, and investigated difference in risk factors associated with growth restriction and inflammation between infants with bronchopulmonary dysplasia and infants with respiratory distress syndrome only.

Results

Infants in studies I and II had reduced risk of respiratory distress syndrome after exposure of antenatal corticosteroids. In term infants an increased risk of low Apgar score, was noticed. Study III showed that preeclampsia was the strongest prenatal risk factor for bronchopulmonary dysplasia. A reduced risk of bronchopulmonary dysplasia associated with diabetes mellitus and gestational diabetes was also found. Study IV showed an increased risk of bronchopulmonary dysplasia associated with long duration of prelabor preterm rupture of membranes, small for gestational age, low Apgar score and resuscitation interventions in the delivery room.

Conclusions

Studies I and II confirmed the benefits of antenatal corticosteroids in preterm infants in a clinical setting and also in infants born late preterm. Except for an increased risk of low Apgar score in term infants, no increased risks of adverse effects were found. The findings from Study III, with preeclampsia as the strongest risk factor and a reduced risk associated with diabetic disorders, suggest that an impaired angiogenesis may contribute to development of bronchopulmonary dysplasia. The findings from Study IV indicate that infants who subsequently develop bronchopulmonary dysplasia were likely to have been exposed to factors causing lung injury and triggering inflammation already during fetal life.

LIST OF PUBLICATIONS

I. Eriksson L, Haglund B, Ewald U, Odlind V, Kieler H. *Short and long-term effects of antenatal corticosteroids assessed in a cohort of 7 827 children born preterm.* Acta Obstet Gynecol Scand. 2009;88(8):933-8.

II. Eriksson L, Haglund B, Ewald U, Odlind V, Kieler H. *Health consequences of prophylactic exposure to antenatal corticosteroids among children born late preterm or term*. Acta Obstet Gynecol Scand. 2012 Dec;91(12):1415-21

III. Eriksson L, Haglund B, Odlind V, Altman Kieler H. *Prenatal inflammatory risk factors for development of bronchopulmonary dysplasia*. Accepted for publication in Pediatric Pulmonology. 2013;

IV. Eriksson L, Haglund B, Ewald U, Odlind V, Altman M, Kieler H. *Perinatal risk factors for bronchopulmonary dysplasia as compared with respiratory distress syndrome.* In manuscript

CONTENTS

LIST OF ABBREVIATIONS

ACS	Antenatal administration of Corticosteroids
ATC	Anatomical Therapeutic Chemical classification
BPD	Bronchopulmonary Dysplasia
CI	Confidence Interval
ECMO	Extracorporeal Membrane Oxygenation
iNO	Inhaled Nitric Oxide
nCPAP	Nasal Continuous Positive Airway Pressure
PDA	Patent Ductus Arteriosus
PIE	Pulmonary Interstitial Emphysema
PNQn	Swedish Perinatal Quality Register Neonatal
PPHN	Persistent Pulmonary Hypertension of the Newborn
PPROM	Preterm Premature Rupture of Membranes
OR	Odds Ratio
RCT	Randomized Controlled Trial
RDS	Respiratory Distress Syndrome
SGA	Small for Gestational Age
TTN	Transient Tachypnea of the Newborn

1 INTRODUCTION

The increased survival rate of infants born preterm is generally attributed to improved interventions for the treatment of respiratory morbidity.[2] Such interventions include antenatal corticosteroids (ACS), postnatal surfactant treatment and gentle ventilation strategies.[2] Whether the beneficial effects of ACS shown in randomized controlled trials (RCT), are equally apparent in a clinical setting of modern neonatal care, is poorly studied.[7] In addition, impaired fetal growth and adverse neurological effects have been reported from both human and animal studies.[8-11] RCTs have not been able to sufficiently confirm or reject suspicions of short or long term adverse effects of ACS.

Despite improved neonatal care, the overall incidence of bronchopulmonary dysplasia (BPD) has not changed over the past decade, probably due to the increased survival of very immature infants.[12] BPD remains the most common complication of very preterm birth, causing long term morbidity and frequent re-admissions to hospital in the first years of life.[13] There is no immediate cure for BPD and treatment is purely symptomatic. Knowledge concerning perinatal risk factors and early identification of infants at risk of BPD should facilitate strategies for preventing this chronic pulmonary disease in infancy.

The overall aim of this thesis was to increase the knowledge of risk factors implicated in the development of BPD and to evaluate the effect of ACS used in a clinical setting of modern neonatal care.

2 BACKGROUND

2.1 DEFINITION AND INCIDENCE OF PRETERM BIRTH

According to the definition recognized by the World Health Organization, preterm birth is defined as birth before 37 gestational weeks. Birth in the interval between gestational weeks 34-36 is usually defined as late preterm. Very preterm birth is birth between 28-33 gestational weeks and extremely preterm birth is defined as birth before 28 gestational weeks.[14]

Six percent of all live born infants in Sweden are born before 37 gestational weeks and 1.2% are born before 33 gestational weeks.[1-2] In societies with advanced medical care the survival rate of infants born at gestational week 28 is approximately 90-95% and at gestational week 24 the survival rate is approximately 50%.[15-17] Recent research suggests that there are two main causes of severe prematurity; infection/inflammation and abnormal vascular/placental development.[18]

2.2 CARDIOPULMONARY MORBIDITY AND RELATED TREATMENT

The preterm infant has multiple organ immaturity, responsible for morbidity and mortality associated with preterm birth.[19-20] In the extremely preterm infant, this organ immaturity includes kidneys with very low blood flow, low glomerular filtration rate, low tubular sodium reabsorption, and an inability to concentrate urine. The intestines have not developed the normal complement of digestive enzymes and peristalsis is not functioning normally. The skin is minimally cornified, resulting in excessive fluid and heat loss and poor barrier function. The brain has not developed good respiratory control and the immune system is naïve, antibody deficient and poorly responsive to infection.[19-20]

2.2.1 Lung development

Except hyperbilirubinemia, the most common morbidity affecting preterm infants is related to the respiratory organ system.[19-20] The pulmonary tissues defining the potential air-blodd barrier in preterm infants are thicker than at term. Fetal lung development could be described in different stages corresponding to gestational weeks.[19-20]

Pseudoglandular stage
During gestational week 9-16 the preacinary airways are formed with sparse occurrence of capillary vessels.

Canalicular stage
During gestational week 16-26 the bronchioli continuously divide into more and smaller canals and the vascular supply increases steadily.[21]
The fetal lung completes branching of the 16 generations of conducting airways by about gestational week 18 and develops respiratory bronchioles that terminate in around 240 000 saccules at gestational week 24-26.[22] The tissue defining the potential air-blood barrier are thicker than at term and pulmonary microvascular development has not fully vascularized the saccular mesenchyme. The enzymes and lamellar bodies

required for surfactant synthesis and secretion are just appearing as some type II cells begin to mature. The fetal lung actively secretes fetal lung fluid and has not yet developed the ion pumps or lymphatics that assist with the clearance of fetal lung fluid. At the end of this period respiration becomes possible with gas exchange between airway and lung capillary vessels. The lung at 24 to 26 gestational weeks is in the late canalicular stage of development.

Saccular stage
The extent of lung development between 24-26 weeks gestation and 32 weeks is substantial. Extensive vasculogenesis occurs within the developing terminal saccules. Secondary crests are formed along with remodeling and loss of interstitial extracellular matrix.[23] At 30 to 32 weeks the lung is in the saccular stage. Alveolarization of the saccular human lung is believed to begin about 32 week's gestation.

Alveolar stage
Although alveoli are present in some infants at 32 weeks gestation, they are not uniformly present until 36 weeks during the alveolar stage of development. Further, only around 20% of the adult alveolar number is present in the human lung at term. The number of alveoli will increase to the age of eight years - from 50 million at birth to about 250 million.[23]

Before birth, the lungs are filled with fluid which contains a high chloride ion concentration. During the last two weeks before birth the amount of surfactant will increase. When respiration begins at birth, most of the lung fluid is rapidly absorbed by the blood and lymph capillaries, while a small amount is expelled via trachea and bronchi during the delivery process. When air is entering the alveoli, during the first breath, the surfactant coat prevents the development of an air-water (blood) interphase, with high surface tension. Without the surfactant layer, the alveoli would collapse during expiration (atelectasis).

2.2.2 Transient tachypnea of the newborn

Transient tachypnea of the newborn (TTN) results from delayed clearance of lung fluid and is a common cause of admission of late preterm or term infants to neonatal intensive care units. [24] The condition is particularly common after elective cesarean delivery.[25-26] TTN often resolves spontaneously within a few days after birth. Conventional treatment involves supplemental oxygen, withholding enteral feeds and administration of intravenous fluids and antibiotics. Rarely, infants require CPAP and mechanical ventilation. Occasionally, some infants develop severe hypoxemia and may require high concentrations of oxygen, but TTN has not been associated with any long term complication.[27]

2.2.3 Respiratory distress syndrome

Respiratory distress syndrome (RDS) is a common disorder in preterm infants. The incidence is inversely related to gestational weeks at birth, 91% of infants born at gestational weeks 23-25, 74% at gestational weeks 28-29 and 52% at gestational weeks 30-31 being affected.[28] RDS is mainly caused by surfactant deficiency resulting in high alveoli surface tension and low lung compliance, leading to alveolar inflammation and

presence of hyaline membrane. In its natural course, symptoms appear shortly after birth and increase in severity over the first two days of life. Clinically, RDS presents with early respiratory distress comprising cyanosis, grunting, retractions, and tachypnoea.[28]

Surfactant replacement therapy is crucial in the management of RDS.[29] Surfactant could be given as a prophylactic or as a rescue therapy, but administration requires intubation.[30] Mechanical ventilation can be life-saving but could also cause lung injury and, wherever possible, nasal CPAP should be used.[28, 31] In order to induce endogenous surfactant production, prophylactic treatment with ACS is recommended to all Swedish women presenting with imminent preterm delivery up to gestational week 32+6.[7, 32] The introduction of ACS, surfactant treatment and new ventilator strategies have resulted in major improvements in the clinical course and outcomes of preterm infants with RDS. For infants with RDS to have best outcomes, it is essential that they have optimal supportive care, including maintenance of normal body temperature, proper fluid management, good nutritional support, management of the ductus arteriosus and support of the circulation to maintain adequate tissue perfusion.[28, 33] However, despite improved treatment strategies, RDS may progress into severe hypoxia, respiratory failure and death or to BPD.

2.2.4 Air leak syndrome

Air leak syndrome includes pulmonary interstitial emphysema (PIE), pneumothorax, pneumomediastinum, pneumopericardium, pneumoperitoneum, subcutaneous emphysema and systemic air embolism. Leakage of gas from the airways into the pulmonary interstitial space is mostly seen as a complication from ventilator therapy, most commonly in preterm infant with fragile and immature lungs.[34-35] The incidence of air leaks in the newborn is inversely related to the birth weight of the infant, especially in very-low-birth-weight infants. When the air leak is asymptomatic and the infant is not mechanically ventilated, there is usually no specific treatment. Emergent needle aspiration and/or tube drainage are necessary in managing tension pneumothorax or pneumopericardium with cardiac tamponade. To prevent air leak syndrome, gentle ventilation with low pressure, low tidal volume, low inspiratory time, high rate, and judicious use of positive end expiratory pressure are the keys to caring for mechanically ventilated infants.

2.2.5 Meconium aspiration syndrome

Meconium aspiration syndrome (MAS) is a common cause of severe respiratory distress in term or near term infants and associated with a significant respiratory morbidity and mortality.[36-37] MAS results from aspiration of meconium during intrauterine gasping or during the first few breaths. Fetal hypoxic stress can stimulate colonic activity, resulting in the passage of meconium and also stimulate fetal gasping and result in meconium aspiration in utero. The pathophysiology of MAS is multifactorial and includes acute airway obstruction, surfactant dysfunction or inactivation, chemical pneumonitis with release of vasoconstrictive and inflammatory mediators and persistent pulmonary hypertension of newborn (PPHN). This disorder can be life threatening, often complicated by respiratory failure, pulmonary air leaks, and PPHN. Approaches to prevent MAS have changed over time with collaboration

between obstetricians and pediatricians forming the foundations for care. The use of surfactant and inhaled nitric oxide (iNO) has led to decreased mortality and reduced the need for extracorporeal membrane oxygenation (ECMO) use.

2.2.6 Persistent Pulmonary Hypertension of the newborn

PPHN is a severe pulmonary disorder which occurs at a rate of one in every 500 live birth.[38] The main cause of pulmonary hypertension in newborn infants is failure of the pulmonary circulation to dilate.[39-40] This syndrome is characterized by sustained elevation of pulmonary vascular resistance, causing extrapulmonary right-to-left shunting of blood across the ductus arteriosus and foramen ovale and severe hypoxemia.[40] Symptoms of severe hypoxemia usually appear directly after birth, but, occasionally, symptoms can develop several hours or even days after birth. There are many unresolved issues regarding the patho-physiology but PPHN is often secondary to other morbidities, such as congenital diaphragm hernia, severe RDS, septicemia, meconium aspiration or asphyxia.[40-41] In addition, exposure to selective serotonin reuptake inhibitors during pregnancy has been shown to increase the risk of PPHN in the infant.[41] Treatment with iNO, a potent selective pulmonary vasodilator has made management of PPHN easier than it was a decade ago. Nevertheless, the mortality rate remains unchanged (10-15%) and 7-20% of the survivors develop long-term sequelae, such as hearing deficit, chronic lung disease, and intracranial bleed.[38, 41]

2.2.7 Patent Ductus Arteriosus

In fetal circulation the ductus arteriosus connects the pulmonary artery with the aorta. In this way, oxygenated blood from the placenta is distributed blood to internal organs, by-passing the lungs. The ductus arteriosus closes rapidly after term birth due to increased arterial blood oxygen tension (PaO_2) and decreased circulating prostaglandin levels. In preterm infants, the ductus arteriosus can sometimes persist and a hemodynamically significant left-to-right shunt will develop. A persistent patent ductus arteriosus (PDA) impairs pulmonary mechanics, prolonging the need for mechanical ventilation and increasing the risk of pulmonary hemorrhage and altering alveolar surface area.[42] Pharmacologic closure of PDA with non-steroid anti-inflammatory drugs (indometacin or ibuprofen) inhibits prostaglandin synthesis and induces ductal constriction. Pharmacological treatment is indicated to prevent the negative effect on pulmonary function and alveolar development.[43-44] In some infants, a surgical closure of PDA is required. Population-based, observational studies suggest that surgical ligation is an independent risk factor for the development of BPD.[45-46] However, it is difficult to determine whether the increased risk is due to the ligation itself and the effect of surgery, or to other factors, such as prolonged left-to-right PDA shunt, which might coexist in infants who require ligation.

2.2.8 Bronchopulmonary dysplasia

BPD is synonymous to chronic lung disease which is a serious disorder affecting preterm infants. See a detailed description in section 2.3 below.

2.3 BRONCHOPULMONARY DYSPLASIA

2.3.1 Incidence

The overall incidence of BPD in preterm newborns has not changed over decades and has been reported to be approximately 20%.[5] In preterm newborns weighing <1500 gram, the incidence of BPD is between 15-47%.[47] In Sweden around 2400 infants were diagnosed with BPD between the years 1988 and 2009.

2.3.2 Definition

Various criteria have been used to diagnose BPD. Traditionally, infants are diagnosed as having BPD if they have been oxygen dependent for at least 28 days after birth.[48] The US National Institutes of Health (NIH) have presented diagnostic criteria that include evaluation of oxygen need at 36 weeks postmenstrual age (gestational age plus chronological age).[49-50] If an infant has been oxygen dependent for at least 28 days but is breathing room air at 36 weeks postmenstrual age, the condition is considered mild BPD. Moderate BPD is defined as a need for <30% O_2 at 36 weeks of postmenstrual age. Severe BPD is defined as need for >30% O_2 (with or without positive pressure ventilation or continuous positive pressure respiratory support).[50-51] To enable a more accurate diagnosis, an oxygen reduction test is used to determine whether supplementary oxygen is still required.[49]

2.3.3 Etiology

Knowledge concerning the etiology of BPD is limited and development is multifactorial.[52] The most important predictor for BPD development is degree of prematurity but a myriad of other fetal and postnatal factors may contribute. [23, 51, 53] The interactive role of pre- and postnatal growth restriction and inflammation (alone or in association with infection) has been suggested to be keystones for BPD pathogenesis.[54-55 56]

High concentrations of inflammatory markers, such as IL-6 and TNF-alpha have been found in cerebrospinal fluid in infants with BPD.[57] One inflammatory mediator that may be central to fetal inflammation and BPD is IL-1. IL-1 is an early acute phase response cytokine in alveolar macrophages and in lung epithelium, but it is also stored as a procytokine in inflammasomes, (i.e. intracellular organelles that have primarily been identified in inflammatory cells).[58-59] In a fetal sheep model of chorioamnionitis, IL-1 mediated most of the lung and systemic inflammation induced by lipopolysaccharide.[60]

However, the exact mechanism responsible for the severe alterations in the pulmonary structure and function of the neonate with BPD is still unknown and biomarkers for BPD are in research focus since prediction of which infant that will develop BPD is so difficult. Genetic factors may also contribute to BPD at multiple levels.

2.3.4 Risk factors

The most important predictor for BPD development is short gestational age but fetal growth restriction, preeclampsia, RDS and mechanical ventilation are other recognized risk factors.[12, 61-63] Pneumonia and other serious infections might also increase risk of

developing BPD but the role of prenatal infection, such as chorioamnionitis, is unclear.[57, 61-62, 64-65]

Moreover, clinical observations indicate that fetal exposure to inflammation may have both detrimental and beneficial effects on the preterm lung.[54] It has been proposed that fetal exposure to chronic inflammation may induce lung maturation and thereby reduce the incidence of RDS but may, on the other hand, promote the development of BPD.[54]

2.3.5 Treatment

Infants with BPD will receive intense supportive care but there is no available medical treatment that can immediately cure BPD. Thus, treatment is purely symptomatic.[47, 66]

Postnatal steroids have been used for many years in the treatment/prevention of BPD based on meta-analyses suggesting decreased rates of BPD with steroid use.[67-70] However, concerns over adverse effects on subsequent negative neuro-developmental outcome have contributed to a significant reduction in routine use of postnatal steroids.[71] The current understanding is that systemic postnatal steroids should be reserved for ventilated infants who cannot be weaned from ventilator support.[72-74] In addition, postnatal steroids given as inhalation have not been shown to reduce outcomes of death or BPD, nor did inhaled postnatal steroids impact on short term outcomes such as failure to extubate, oxygen dependency or total duration of mechanical ventilation.[72]

Inhaled NO (iNO) is primarily used for the treatment of PPHN but may also be used as prevention of BPD. Treatment with iNO may improve ventilation-perfusion mismatch and oxygenation, lower pulmonary arterial pressure, reduce lung inflammation and thereby attenuate the pathophysiology of respiratory distress syndrome. However, the National Institute of child Health and Human development, USA (NICHD) trial of inhaled nitric oxide in preterm infants did not show a difference in the primary outcome of BPD and death between controls and those having received iNO.[75] Therefore, iNO remains a controversial treatment for premature infants with severe respiratory failure.[76-77]

Recent experience in neonatology suggests that combining less invasive care strategies that avoid excessive oxygen and mechanic ventilation, decreasing postnatal infection and optimize nutrition, may decrease the incidence and severity of BPD.[31, 78-79]

2.3.6 Clinical characteristics of BPD

The clinical characteristics and the natural history of infants affected by BPD have changed considerably over the last decades, probably due to advances in perinatal care and neonatal respiratory therapy. In the past, infants developed BPD after severe respiratory failure, frequently compounded by a PDA, PIE and/or infection necessitating high-pressure ventilation and supplementary oxygen concentration.[80] The former severe BPD has been replaced by a clinically milder form, without or with mild RDS in the first days of life, that responds rapidly to surfactant therapy but requires prolonged ventilator support because of poor respiratory effort.[21]

2.3.7 Long term outcome of BPD

Patients diagnosed with BPD who survive the first month of life have a 30% increased risk of dying in the first year of life.[66, 81] The main causes of death in these patients are respiratory failure, systemic infections and cor-pulmonale. Patients with BPD have frequent respiratory infections during the first two years of life and an increased frequency of bronchial hyperactivity.[66, 81]

Affected children require frequent readmissions to hospital, and, although lung growth and remodeling result in progressive improvement in lung function, airflow abnormalities may remain. After dismissal from hospital, most infants with BPD will require continued medication, breathing treatments and oxygen during their first year of life. The lung problems can linger into childhood, adolescence and even into adult ages.[51, 82-83] Neurological development is affected in approximately 40% of patients with BPD.[84]

2.4 ANTENATAL CORTICOSTEROIDS

In 1972, Liggins and Howie published a landmark article demonstrating that ACS significantly reduced the frequency of RDS and neonatal mortality. [85-86] Thereafter, ACS treatment was gradually introduced worldwide and ACS treatment is now an established intervention in the prevention of RDS in premature infants.

In fetal lung maturation, ACS accelerates the effect of endogenous corticosteroids and induces the production of all the components of the surfactant system. ACS may also facilitate clearance of lung fluid by activating the epithelial sodium channels and increasing ion transport, but this mechanism has not been confirmed. The net results are improved lung function, better response to surfactant and improved survival.[48] Other proposed beneficial effects of ACS under debate, are liver maturation and reduction of the risk of neonatal jaundice requiring phototherapy. In addition to reduction of RDS, RCTs have shown that ACS treatment also reduces the risk of neonatal death, intraventricular hemorrhage and necrotizing enterocolitis.[7]

Current European Consensus Guidelines recommends ACS therapy in all pregnancies with imminent preterm delivery before 35 weeks' gestation and concludes that there is strong evidence for the role of a single course of antenatal steroids in RDS prevention, but the potential benefit and long-term safety of repeated courses is unclear.[28] Betamethasone and dexamethasone have both been used to enhance fetal lung maturity. Previous observational cohort studies have suggested an increased rate of cystic periventricular leucomalacia associated with dexamethasone but this could not be confirmed in a Cochrane Review.[87] At present, there is no definite recommendation on the choice of steroid.

In Sweden, the administration of ACS was gradually introduced during a 20 year period with great differences in starting year of routine use between different maternity wards.[88] It is estimated that by year 1997 all maternity wards in Sweden had introduced this routine. Currently, ACS is offered to all women presenting with imminent preterm delivery before gestational week 33.[28]

In contrast to the beneficial effect of ACS on RDS, the effects on BPD have been less obvious. Some studies report positive effects, whereas others have seen no effects or even negative effects.[89-92]

3 AIMS

The overall aim of this thesis was to investigate pulmonary disease in infancy, in particular perinatal inflammatory risk factors for pulmonary disease with specific emphasis on the development of BPD. Further, the aim was to study prophylaxis of pulmonary disease in the neonate, with specific emphasis on the effect of ACS.

The specific aims of the included studies were

- To assess whether ACS treatment affects neonatal mortality, RDS, intraventricular hemorrhage, retinopathy of prematurity, BPD, and long-term outcome such as epilepsy and cerebral palsy in children born before gestational week 34 (Study I);

- To investigate whether exposure of ACS before gestational week 34 was associated with reduced risk of RDS and BPD in infants born late preterm or term, and if there were any increased risks of deviant fetal growth, other adverse neonatal outcomes, or epilepsy, cerebral palsy or diabetes during childhood (Study II);

- To identify prenatal risk factors for BPD, focusing on inflammation, and to investigate whether maternal inflammatory diseases, pregnancy related diseases and related pharmacological treatment during pregnancy, may affect the risk of BPD development in preterm infants (study III);

- To identify factors that may affect the development of BPD in infants by comparing perinatal growth and factors related to inflammation between infants with BPD and infants with RDS only, and also comparing the occurrence of other factors related to these two disease entities (Study IV).

4 METHODS

Study designs

All papers in this thesis are based on newborn infants, born during a period of over thirty years; from 1976 through 2010 in Sweden. The majority was born preterm before 37 gestational weeks. Two different study designs were used; cohort studies (studies I-III) and a case-control study (study IV).

Data sources

The Swedish national health registers (*the Medical Birth Register, the Patient Register, the Cause of Death Register and the Prescribed Drug Register*) were the primary sources of data in studies I-III, whereas *the Swedish Perinatal Quality Register neonatal* (PNQn) was the primary source of data in study IV. Diagnoses in all the registers are classified and recorded by the treating physician according to the International Classification of Diseases (ICD) at the time of discharge, according to current ICD version. All registers include the national registration number assigned to each Swedish resident, which enabled us to merge data from different registers for a specific infant.

The Medical Birth Register

The Swedish Medical Birth Register was established 1973 and contains information on more than 99% of all births in Sweden, including data on the mother, pregnancy, delivery and neonate, and diagnoses at discharge from the hospital.[93] Since 1994, information on drug use from antenatal care has been recorded in the Swedish Medical Birth Register and subsequently, this information is converted into the World Health Organization Anatomical Therapeutic Chemical (ATC) classification.

The Prescribed Drug Register

Information on dispensed drugs during pregnancy is found in The Prescribed Drug Register which started in July 2005. Drugs in the register are recorded according to ATC. The Prescribed Drug Register contains information with unique patient identifiers for all prescriptions dispensed to the whole population of Sweden. For prescribed drugs, the register includes data on dispensed item, substance, brand name, formulation, package size, dispensed amount, dosage, expenditure and reimbursement. The register does not include information on over-the-counter medications nor on drug used or administered in hospitals. Information on drug treatment during a hospital stay is noted in the individual medical record, but this information is currently not forwarded to the Prescribed Drug Register.[94]

The Patient Register

The Patient Register includes dates of each hospital admission and discharge, as well as main discharge diagnosis and secondary diagnoses. From 2001 information on outpatient care is also covered.[95]

The Cause of Death Register

The Cause of Death Register contains dates and causes of all deaths among Swedish residents.

The Swedish Perinatal Quality Register neonatal
The PNQn was created in 2000 as a local hospital register. From January 2008 and onwards, PNQn contains data on all infants admitted to the neonatal units in Sweden. Data in the register is obtained from standardized questionnaires prospectively filled by the physicians during neonatal care and includes information on resuscitation, ventilator support, specified pharmacological treatment, other interventions and morbidity.

4.1 STUDY POPULATION IN STUDIES I AND II

General considerations
With the overall aim of evaluating the comprehensive health consequences of the widespread routine treatment with ACS to women with imminent preterm delivery, studies I and II were performed. Further, because adverse effects are typically much less common than the intended positive effects of drugs, RCTs conducted to evaluate prophylactic interventions, are seldom large enough to provide an adequate assessment of drug safety. Therefore, the primary objective when designing studies I and II was to evaluate short and long-term adverse effects of ACS, in the population that actually received the treatment in the real clinical setting.

Study populations
The cohorts in studies I and II were identified through the Swedish Medical Birth register during the years 1976 through 1997. The time period mirrors the years during which ACS treatment was established as routine prophylaxis in Sweden.

Study I included live-born singleton infants, born before gestational week 34. The gestational age limit was based on the results from questionnaires and telephone interviews with physicians at Swedish maternity wards (performed prior to studies I and II), which showed that several Swedish hospitals administered corticosteroids up to gestational week 34.[88] In total, 7 827 infants were included and followed up to 9 years of age or to date of death.

Study II included singleton infants, stillborn or live-born to women who were hospitalized due to imminent preterm delivery before 34 gestational weeks, but who eventually delivered late preterm or term. In total, 11 873 infants were included and, of these, 3 550 were born late preterm (gestational weeks 34 - 36), and 8 323 were born at term (gestational week 37 or later). The children were followed to their 11th birthday or date of death.

4.2 EXPOSURE ALGORITHM IN STUDIES I AND II

Studies I and II used the same exposure algorithm to categorize an infant into the exposed or the unexposed cohort.

Exposure status at the hospital level
In Sweden, routine administration of ACS was gradually introduced over a 20 year time period. There was a great variability in starting year between different maternity wards, which opened the opportunity to use a novel exposure algorithm. If an infant was born at a maternity ward which routinely offered ACS prophylaxis, the infant was

classified as exposed. Conversely, if an infant was born at a maternity ward that had not established this intervention, the infant was classified as unexposed. In study II (in which delivery occurred weeks or months after ACS exposure) the exposure algorithm was related to the time period when the mothers were hospitalized due to imminent preterm delivery.

To identify hospital routines on ACS prophylaxis, we combined information obtained from written questionnaires to hospitals, telephone interviews with physicians working at the maternity wards, and pharmacy sales statistics.

Assessment of hospital routines

A questionnaire was sent to all 52 departments of obstetrics and gynecology in Sweden, including questions on starting year, dose, type, route of administration and gestational age limits for prophylactic ACS treatment. Telephone interviews with physicians at those departments, asking similar questions as in the questionnaires, were also performed. Pharmacy sales statistics were thereafter used to confirm or discard the information obtained from physicians and questionnaires. Purchased large quantities of corticosteroids strengthened information on routine use. Low or no purchased quantities confirmed that ACS prophylaxis was not an established intervention.

Pharmacy sales statistics related to number of preterm deliveries

Information on annual sale volumes of betamethasone and dexamethasone from 1976 through1997 for each of the 52 departments of obstetrics and gynecology in Sweden was abstracted from the Swedish Pharmacy Company. Purchased annual volumes were compared with the annual number of preterm deliveries at that specific department. A quota was calculated in which the department's annual corticosteroid volumes were divided by their annual number of preterm deliveries.

The recommended prophylactic ACS dose, in case of imminent preterm delivery, is 24 mg. Therefore the "annual quota cut-point" was set to 20 mg as a limit for routine use. Thus, if the calculated quota at a specific department exceeded 20 mg per preterm delivery, all infants born at that hospital that year were regarded as exposed.

If the calculated annual quota was less than 10 mg per preterm delivery, all infants born at that hospital that year were regarded as unexposed to ACS. If the calculated quota was between 10 mg an 20 mg data were regarded as invalid and routine use of ACS could not be categorized. Therefore, all children born at that specific department that year were excluded from analyses. Further, to reduce the effect that a single delivery might have on the calculations, all infants born at hospitals with fewer than 10 preterm deliveries per year were excluded.

Validation of exposure algorithm

To validate our method for estimation of exposure on hospital level, a detailed analysis of medical records on a random sample of 120 women was performed in study II. We retrieved copies of original medical records, which were thoroughly examined regarding information on ACS administration. The exposure estimation at hospital level was thereafter compared with the information on ACS as noted in the medical records, using medical records as reference method.

The quality of exposure classification was evaluated by sensitivity and specificity. Sensitivity was the concept used to describe the probability that an individual who was actually exposed to ACS, would also be classified as exposed. Specificity was the concept used to describe the probability that an individual who was not exposed to ACS, would also be classified as unexposed.

Definition of sensitivity and specificity

Sensitivity and specificity displayed by a 2*2 table

Medical record=Reference method	Exposed according to exposure algorithm	Unexposed according to exposure algorithm
Exposed according to medical record	True positive (TP)	False negative (FN)
Unexposed according to medical record	False positive (FP)	True negative (TN)

Sensitivity = TP/(TP + FN)
Specificity = TN/(TN + FP)

4.3 OUTCOMES IN STUDIES I AND II

Outcome measures in both studies included Apgar score < 7 at five minutes, RDS, BPD, epilepsy and cerebral palsy. In study I, neonatal death was categorized into early (0-6 days), late (7-27 days) and post (28-364 days) neonatal death. In study II, neonatal death was instead evaluated as stillbirth or overall mortality during the first year of life.

The effect on intraventricular hemorrhage and retinopathy of prematurity was only evaluated in study I, whereas effect on childhood diabetes was only evaluated in study II. Further, study II evaluated the effect on infant size including birth weight, birth length and head circumference in relation to gestational age. Small for gestational age (SGA) was defined as a birth weight/ length or head circumference of two standard deviations or more below the mean according to sex specific fetal growth curves [96].

Information on maternal and infant characteristics and short term outcome such as infant size, Apgar score, RDS, intraventricular hemorrhage and retinopathy of prematurity were gathered from the Medical Birth Register. Data on long term outcome such as epilepsy, cerebral palsy and childhood diabetes were collected from the Patient Register. Information on stillbirth and neonatal death were collected from the Cause of Death Register.

4.4 STUDY POPULATION IN STUDIES III AND IV

General considerations

With the overall aim of increasing the knowledge of risk factors for the development of BPD, studies III and IV were performed. Study III was explorative and used the cohort study design, including all preterm infants born over a 20-year period in Sweden, evaluating a broad range of potential risk factors focusing on inflammation. Study IV used the case-control study design to address differences in perinatal growth and inflammatory risk factors between infants diagnosed with BPD and RDS, respectively. Included infants were born extremely and very preterm in an era of modern neonatal care.

Study populations

The cohort in study III was identified through the Swedish Medical Birth Register and consisted of all singleton infants, live-born before 37 gestational weeks and surviving 28 days or beyond during the years 1988 through 2009 in Sweden. In total, 106 339 infants were included of which 3 388 were born extremely preterm (between gestational weeks 22 to 27).

Cases and controls in study IV were identified through the PNQn and included preterm infants, live born before 33 completed gestational weeks, during the years 2005 through 2010 in Sweden. All infants were admitted to a neonatal intensive care unit at birth, and survived 28 days or beyond. Cases were infants who were oxygen dependent at 36 weeks post menstrual age and thereby were considered to have BPD.[49] Controls were infants diagnosed with RDS, but with no oxygen dependence at 36 weeks post menstrual age. The controls were matched to cases according to gestational age in gestational weeks at birth. To avoid misclassification of cases and inclusion of infants with oxygen dependence due to other causes than BPD, all infants born with congenital heart and pulmonary malformations were excluded.

4.5 OUTCOME AND RISKFACTORS IN STUDIES III AND IV

Study III

Outcome was defined as a diagnosis of BPD during the first year of life, recorded in the Patient Register and/or the Cause of Death Register and was assessed by gestational age strata for all infants born preterm (< 37 gestational weeks) and for infants born extremely preterm (<28 gestational weeks).

Potential risk factors were categorized into three main areas: maternal chronic inflammatory disease, pregnancy related disease and drugs related to treatment of inflammation/ infection during pregnancy.

Maternal chronic inflammatory diseases served as indicators for maternal systemic inflammation and included Crohn´s disease, ulcerative colitis, psoriatic arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, arthritis associated with Crohn´s disease or ulcerative colitis, sarcoidosis, Mb Sjogren, and psoriasis. These diagnoses were searched for in the Patient register, starting 10 years prior to delivery. If a maternal diagnosis was registered only in connection with outpatient care, 2 outpatient visits were required to

qualify as indicator of maternal systemic inflammation. Information on maternal asthma, diabetes, endometriosis and psychiatric diseases was also obtained. Maternal psychiatric diseases served as a reference marker for increased risk of preterm birth but not associated with inflammation. In all analyses, maternal chronic inflammatory diseases were analyzed as one entity.

Information on pregnancy related diseases was extracted from the Medical Birth Register and included gestational diabetes, preeclampsia related disorders (pregnancy induced hypertension, preeclampsia, eclampsia) and chorioamnionitis related disorders (amnionitis, chorioamnionitis, prelabor preterm rupture of membranes). Maternal genito-urinary infections were categorized into upper urinary tract infections (pyelitis) or lower urinary tract infections (cystitis). In all analyses, the preeclampsia related disorders were analyzed as one entity and, similarly, all the chorioamnionitis related disorders were analyzed as one entity.

Estimation of fetal exposure to drugs related to treatment of inflammation/infection during pregnancy was based on prescriptions dispensed to the mothers during pregnancy. Anatomical Therapeutic Chemical classification (ATC) codes for methotrexate, other disease modifying antirheumatic drugs, TNF-alpha inhibitors, non-steroid anti-inflammatory drugs, corticosteroids, anti-diabetics and antibiotics were searched for in the Prescribed Drug Register. As recording in the Prescribed Drug Register started in July 2005, drug exposure was assessed in infants born to mothers who had their last menstrual period 90 days after the 1st of July 2005.
Exposure to methotrexate, other disease modifying antirheumatic drugs and TNF-alpha inhibitors were analyzed as one entity, whereas, non-steroid anti-inflammatory drugs, corticosteroids and antibiotics were analyzed separately.

Study IV
Cases with BPD and controls with RDS were compared concerning prenatal exposure of factors related to inflammation and growth restriction, risk factors in relation to delivery and resuscitation in the delivery room, co-morbidity and postnatal infections, ventilation strategies and pharmacological treatments.

As prenatal risk factors related to inflammation or growth restriction, we assessed SGA, use of ACS, prelabor preterm rupture of membranes (PPROM), preeclampsia related disorders, gestational diabetes, maternal smoking in early pregnancy and season of birth as a proxy for exposure to maternal virus infections (winter or summer).

As risk factors in relation to delivery and resuscitation in the delivery room, we assessed the effect of mode of delivery (vaginal or cesarean delivery), Apgar score at 5 minutes, overall resuscitation in the delivery room and interventions as part of resuscitation.

As co-morbidity and postnatal infections, we included PDA and related treatment (pharmacological or surgical ligation), PPHN, PIE and pneumothorax were included as co-morbidity and postnatal infections. The effect of culture positive early and late onset infections was also evaluated. Early onset infection was defined as start of symptoms

before 72 hours after birth and late onset was start of symptoms 72 hours after birth or later.

The association between BPD and ventilation strategies (nasal continuous positive airway pressure [nCPAP] and mechanical ventilation) was assessed. The evaluation of pharmacological treatments included surfactant, iNO and postnatal steroids.

4.6 STATISTICAL ANALYSES

In all four studies, the results are presented as odds ratios (OR) and 95% confidence intervals (95%CI). In studies I-III, unconditional logistic regression models were used to evaluate associations between exposure and outcome. In study IV conditional logistic regression was used to evaluate the associations. Logistic regression provides a flexible means of analyzing the association between a binary outcome and a number of exposure variables.[97] Conditional logistic regression is a variant of logistic regression in which cases are only compared to controls in the same matched set. Additional confounders may be included in the model and there is no restriction on the numbers of cases and controls in each matched set.[97]

Odds and Odds ratio

The odds of the event are the ratio of the probability of the event happening divided by the probability of the event not happening.

The odds ratio is estimated by:

$$\text{OR} = \frac{\text{odds of disease in exposed group}}{\text{odds of disease in the unexposed group}} = \frac{d_1/h_1}{d_0/h_0} = \frac{d_1 * h_0}{d_0 * h_1}$$

d_1=diseased in the exposed group d_0=diseased in the unexposed group
h_1=healthy in the exposed group h_0= healthy in the unexposed group

By the 2*2 table the OR can be expressed as = $\frac{a/b}{c/d}$

Drug/risk factor	Cases/diseased	Controls/healthy
Exposed	a	b
Unexposed	c	d

The OR in a cohort study could be interpreted as a Risk Ratio (RR) when the disease is rare (prevalence <10%). However, the OR is always further away from 1 than the corresponding RR.[97-98]

An OR of 1 indicates no association between the exposure and outcome. A greater value than 1 indicates an increased odds of having the disease among exposed subjects. A value below 1 indicates decreased odds of having the disease among exposed subjects.[97]

Logistic Regression

Logistic regression allows the analysis of dichotomous or binary outcomes with two mutually exclusive levels and the ability to adjust for multiple predictors.[99-100] Thus, logistic regression quantifies the association between a risk factor or treatment and a disease, after adjusting for other variables.[99-100]

Logistic regression results are presented by odds ratios because these are the natural estimates from the model. The logistic regression model takes the natural logarithm of the odds as a regression function of the predictors. With one predictor, X, this takes the form $\ln[\text{odds}(Y=1)]=\beta_0+\beta_1 X$, where ln stands for the natural logarithm, Y is the outcome and Y=1 when the event happens (versus Y=0 when it does not), β_0 is the intercept term, and β_1 represent the regression coefficient, the change in the logarithm of the odds of the event with a 1-unit change in the predictor X.[99-100]

Conditional Logistic Regression

Matching in case-control studies could be done by frequency matching or individual matching.[97] When analyzing individually matched case-control studies we wish to control for confounding variables, additional to those matched for in the design. This is done by *conditional* logistic regression, a variant of logistic regressions in which cases are only compared to controls in the same matched set. Additional confounders may be included in the model and there is no restriction on the numbers of cases and controls in each matched set.

Confounders in studies I and II

In studies I and II, exposure status was estimated at the hospital level. As a true confounder should be related to both exposure and outcome, we adjusted for confounding factors that could interfere with both hospital of birth and outcome. Therefore, year of birth, hospital level and gestational age at birth (study I) and pregnancy duration (gestational age) at first hospital admittance by second degree polynomial (study II) were identified as potential confounders and adjusted for in the statistical analyses.

Confounders in study III

The following potential confounders were adjusted for: maternal age, birth order, hospital level, smoking habits, year of birth and if the mother was born in a Nordic country. In addition, we adjusted for birth weight by gestational age (i.e. small for gestational age [SGA], large for gestational age [LGA]) when testing the association between BPD and preeclampsia related disorders, diabetes mellitus and gestational diabetes.

Since logistic regression is based on the assumption that each subject is selected independently of the others and observations are not independent in women who delivered more than once during the study period, we calculated estimates using clustered data in the generalized estimation equation method.

Confounders in study IV

The following potential confounding factors were adjusted for: maternal age, birth order, multiple births and gestational age as additional days in each gestational week.

In additional stratified analyses, we assessed whether preeclampsia modified the association between SGA and BPD, by including both preeclampsia and SGA in the model as an interaction term. A p-value below 0.05 was considered statistically significant in comparative analyses.

5 RESULTS

5.1 STUDIES I AND II

The cohort in study I consisted of 7 827 infants of which 5 632 were estimated as exposed based on the exposure algorithm. The majority of infants were born moderately preterm and 17% of the exposed and 15% of the unexposed infants were born before 29 weeks of gestation. As this was an observational study, the age distribution in the cohort mirrored the actual occurrence in real life with a majority of infants being born after 29 weeks of gestation. Less than 5% were born at a primary level hospital (local hospital). There were slightly more male than female infants in both the exposed (55 %) and the unexposed (56 %) cohort.

The cohort in study II consisted of 11 873 infants, of which 8 620 infants were estimated as exposed, based on the exposure algorithm. The majority, 8 323 infants, were born at term (gestational week 37 or later) and 3 550 infants were born late preterm (gestational weeks 34 to 36).

After adjusting for potential confounders, we found that exposure to ACS before gestational week 34 was associated with a reduced risk of RDS in all gestational age groups. Of those infants born from gestational week 34 or later, 136 infants were diagnosed with RDS and the majority (n=125) was found among those born late preterm.

Table 1. Antenatal exposure of corticosteroids before gestational week 34 and the risk of respiratory distress syndrome by gestational age at birth (OR=odds ratio, CI=confidence interval).

Gestational age at birth (weeks)	Adjusted OR	Adjusted 95% CI
<29	0.76	0.56 – 1.02
<34	0.80	0.70 – 0.92
≥34	0.50	0.40 – 0.80

Adjusted for: Level of hospital, birth year, gestational age at birth or pregnancy length by exposure

With the exception of an increased risk of low Apgar score, most pronounced in infants born at term, no significant short or long term adverse effects were found. In study II the number of BPD events was too few to allow for meaningful analysis.

Table 2. Antenatal exposure of corticosteroids before gestational week 34 and the risk of Apgar score <7 at 5 minutes by gestational age at birth (OR=odds ratio, CI=confidence interval).

Gestational age at birth (weeks)	Adjusted OR	Adjusted 95% CI
<34	1.02	0.88 – 1.20
34-36	1.31	0.83 – 2.07
≥37	1.60	1.01 – 2.54

Adjusted for: Level of hospital, birth year, gestational age at birth or pregnancy length by exposure

Among infants born before gestational weeks 34 (study I), male infants exposed to ACS had an imprecise risk increase of epilepsy whereas females had a reduced risk (OR 1.74, 95% CI: 0.85-3.55 vs. OR 0.50, 95%CI: 0.25-1.03, respectively, p<0.0141). Among infants born from gestational weeks 34 or later (study II) there was no difference in the risk of epilepsy between male (OR 0.94, 95% CI 0.45 – 1.95) and female (OR 0.77, 95% CI 0.29 – 2.05) infants.

5.2 STUDY III

The study cohort consisted of 106 339 infants. In total, 2 115 infants were diagnosed with BPD of whom 1 393 (66%) were born before 28 gestational weeks.

The mothers of infants with BPD were older, had more often a chronic inflammatory disease or a pregnancy related disorder and were more often delivered by cesarean section. There were more boys than girls in our study cohort and BPD was more common among boys.

Preeclampsia related disorders were associated with a 2-fold risk increase of BPD in infants born before gestational week 37. In infants born before 28 gestational weeks, a 30% risk increased was found. The risk estimate was reduced and became imprecise when birth weight related to gestational age (SGA) was included in the model (OR 1.23, 95% CI 0.92 - 2.45).

Table 3. Preeclampsia related disorders (preeclampsia, eclampsia and pregnancy induced hypertension) and the risk of bronchopulmonary dysplasia in the infant (OR=odds ratio, CI=confidence interval).

Gestational age at birth (weeks)	Crude OR	Crude 95%CI	Adjusted OR	Adjusted 95%CI
<28	1.43	1.20 – 1.70	1.33	1.08 – 1.64
<37	2.05	1.85 – 2.26	2.04	1.83 – 2.29

Adjusted for: maternal age in 5-year strata, birth order, hospital level, smoking habits, year of birth and mother born in a Nordic country.

Chorioamnionitis related disorders and lower urinary tract infections were associated with an increased risk of BPD in all infants but not in infants born before gestational week 28. No association was seen for upper urinary tract infections.

Table 4. Chorioamnionitis related disorders (amnionitis, chorioamnionitis and prelabor preterm rupture of membranes) and Lower urinary tract infection and the risk of bronchopulmonary dysplasia in the infant (OR=odds ratio, CI=confidence interval).

Gestational age at birth (weeks)	Crude OR	Crude 95%CI	Adjusted OR	Adjusted 95%CI
Chorioamnionitis related disorders				
<28	1.00	0.86 – 1.15	0.97	0.81 – 1.15
<37	1.59	1.44 – 1.74	1.33	1.19 – 1.48
Lower urinary tract infection				
<28	0.84	0.52 – 1.38	1.03	0.59 – 1.79
<37	1.71	1.23 – 2.37	1.71	1.19 – 2.45

Adjusted for: maternal age in 5-year strata, birth order, hospital level, smoking habits, year of birth and mother born in a Nordic country.

Maternal diabetes mellitus was associated with a reduced risk of BPD when assessed in infants born before gestational week 37, but this association was not significant in infants born before gestational week 28. Adjustment for birth weight in relation to gestational age (SGA/LGA) did not influence the results. A similar pattern was found for gestational diabetes, with reduced risks when analyzed in all infants, but with a non-significant reduction among extremely preterm infants. In a subgroup analysis, including all mothers with available information on insulin treatment and their infants, we found associations suggesting reduced risk of BPD in infants of mothers with gestational diabetes with insulin as well as without insulin treatment.

Table 5. Diabetes mellitus and gestational diabetes and the risk of bronchopulmonary dysplasia in the infant (OR=odds ratio, CI=confidence interval).

Gestational age at birth (weeks)	Crude OR	Crude 95%CI	Adjusted OR	Adjusted 95%CI
Diabetes mellitus				
<28	0.64	0.29 – 1.41	0.59	0.24 – 1.41
<37	0.67	0.46 – 0.99	0.64	0.42 – 0.97
Gestational diabetes				
<28	0.86	0.37 – 1.97	0.55	0.22 – 1.37
<37	0.49	0.29 – 0.85	0.36	0.20 – 0.65

Adjusted for: maternal age in 5-year strata, birth order, hospital level, smoking habits, year of birth and mother born in a Nordic country.

Data on maternal drug exposure during pregnancy was available for 28% of infants with BPD and for 16% for infants without BPD. Maternal antibiotics were associated with a reduced risk of BPD in all infants (OR 0.70, 95% CI 0.52 – 0.96) and in infants born extremely preterm (OR 0.52, 95% CI 0.30 – 0.90). No increased risk associated with maternal drugs used for chronic inflammatory diseases could be detected.

No increased risk associated with asthma, psychiatric disorders or chronic inflammatory diseases were found, but an imprecise risk increase associated with endometriosis was seen (OR 2.28, 95% CI 0.96 – 5.42).

5.3 STUDY IV

The study population consisted of 2271 infants, of whom 667 were identified as cases with BPD and 1604 as controls with RDS only. Of the BPD cases, 84% had a prior diagnosis of RDS.

Prenatal risk factors related to inflammation or growth restriction
Infants born to women with duration of PPROM more than one week had a more than 3-fold risk increased of BPD. Twenty-eight percent of the cases and 19% of the controls were SGA for birth weight. Factors SGA for length and SGA for birth weight were associated with a 50% to 100% increased risk of BPD.
The majority of mothers of both cases and controls had received prophylactic treatment with ACS. For most infants, there was no available information on the exact time point of administration. Where information was available, ACS given more than 8 hours prior to delivery did not affect the risk of BPD and neither did season of birth, maternal smoking in early pregnancy, preeclampsia related disorders or gestational diabetes. In the stratified analyses, SGA among infants born to mothers with preeclampsia generated a lower adjusted OR of 2.04 (95% CI 1.47-2.83) than SGA without preeclampsia (adjusted OR 3.74, 95% CI 2.61-5.35); a statistically significant difference ($p = 0.008$).

Table 6. Prenatal risk factors related to inflammation or growth restriction and the risk of bronchopulmonary dysplasia in infants born <33 gestational weeks, from 2005 to 2010 in Sweden (OR=odds ratio, CI=confidence interval).

Prenatal risk factors	Crude OR	Crude 95%CI	Adjusted OR	Adjusted 95%CI
Antenatal corticosteroids				
>8h prior to delivery	1.11	0.79 – 1.57	1.13	0.80 – 1.60
Given, but time point missing	1.07	0.70 – 1.44	1.10	0.81 – 1.49
PPROM (time before delivery)				
24h to ≤ 1 week	0.75	0.52 – 1.09	0.76	0.52 – 1.12
> 1 week	3.18	2.07 – 4.89	3.26	2.11 – 5.02
Preeclampsia related disorders	1.14	0.89 – 1.48	1.12	0.86 – 1.46
Gestational diabetes	1.03	0.51 – 2.07	0.97	0.48 – 1.97
Smoking in early pregnancy	0.77	0.52 – 1.16	0.84	0.55 – 1.27
Small for gestational age				
Birth weight	2.57	2.00 – 3.35	2.66	2.05 – 3.44
Length	1.49	1.11 – 1.99	1.49	1.11 – 2.00
Head circumference	1.06	0.78 – 1.43	1.05	0.78 – 1.42

Controls with RDS only were matched to cases with BPD according to gestational age by weeks. Conditional logistic regression was performed with adjustment for maternal age in 5-year age groups, birth order, multiple births, and gestational age by additional days in gestational weeks. ACS=Antenatal Corticosteroids, PPROM=Prelabor Preterm Rupture of Membranes, Preeclampsia related disorders=pregnancy induced hypertension, preeclampsia or eclampsia, SGA=Small for Gestational Age

Risk factors in relation to delivery and resuscitation in the delivery room

Low Apgar score was found in 32% of cases and 15% of controls and increased the risk of BPD by 35%. Overall resuscitation in the delivery room was required by 91% of cases and 77% of the controls. Intubation was required by 56% of the cases and 19% of the controls, corresponding to a 53% increased risk of BPD among those requiring intubation. Chest compressions were required by 5.0% of cases and 2% of controls, corresponding to a 2-fold risk increase of BPD. Cesarean delivery did not affect the risk of BPD.

Table 7. Risk factors evaluated at delivery and resuscitation in the delivery room, and the risk of bronchopulmonary dysplasia in infants born <33 gestational weeks, from 2005 to 2010 in Sweden (OR=odds ratio, CI=confidence interval).

Risk factors at delivery and resuscitation	Crude OR	Crude 95%CI	Adjusted OR	Adjusted 95%CI
Cesarean Section, gestational age at birth (weeks)				
<27	1.25	0.87 -1.78	1.17	0.80 – 1.73
≥27	1.41	1.00 – 1.89	1.38	0.97 – 1.95
Length of resuscitation				
<10 minutes	1.22	0.83 – 1.79	1.17	0.80 – 1.73
≥10 minutes	1.43	1.01 – 2.02	1.39	0.98 – 1.96
Supplemental Oxygen	1.01	0.81 – 1.28	1.01	0.80 – 1.27
Intubation	1.55	1.20 – 2.00	1.53	1.18 – 1.97
Chest Compressions	1.93	1.09 – 3.41	2.04	1.15 – 3.63
Apgar <7 at 5 minutes	1.37	1.05 – 1.78	1.35	1.03 – 1.77

Controls with RDS only were matched to cases with BPD according to gestational age by weeks. Conditional logistic regression was performed with adjustment for maternal age in 5-year age groups, birth order, multiple births, and gestational age by additional days in gestational weeks. GW=Gestational Week.

Cardiopulmonary co-morbidity and postnatal infections

PDA was present in 64% of the cases and in 25% of the controls, corresponding to an increased risk of BPD by 71%. Pharmacological treatment for PDA was given to 49% of cases and 17% of the controls. Surgical ligation was required by 25% of cases and 3% of controls. Of those surgically treated, 69% had received pharmacological treatment prior to surgery. The highest odds ratio related to PDA was seen in infants requiring surgical ligation, corresponding to more than 3-fold increased a risk of BPD.

PPHN, PIE and pneumothorax occurred more often among cases than controls and were associated with a 2- to 5-fold increased risk of BPD. Mechanical ventilation as respiratory support was used in 81% of infants with PIE and in 75% of infants with pneumothorax.

Culture positive late onset infections were more frequent among cases than controls. One episode of late onset infection increased the risk of BPD by 68% whereas having 2 or more episodes more than doubled the risk of BPD. The occurrence of a culture positive early onset infection was overall similar between cases and controls and did not affect the risk of BPD.

Table 8. Cardiopulmonary co-morbidity and postnatal infections and the risk of bronchopulmonary dysplasia in infants born <33 gestational weeks, from 2005 to 2010 in Sweden (OR=odds ratio, CI=confidence interval).

Cardiopulmonary co-morbidity and postnatal infections	Crude OR	Crude 95%CI	Adjusted OR	Adjusted 95%CI
Patent Ductus Arteriosus				
Overall diagnosis	1.66	1.30 – 2.11	1.71	1.33 – 2.18
Pharmacological treatment	1.46	1.14 – 1.86	1.48	1.15 – 1.90
Surgical ligation	3.56	2.41 – 5.26	3.74	2.52 – 5.55
Persistent Pulmonary Hypertension	5.40	3.02 – 9.65	5.40	3.01 – 9.70
Pulmonary Interstitially Emphysema	2.51	1.27 – 4.97	2.54	1.28 – 5.07
Pneumothorax	3.02	1.89 – 4.80	2.99	1.88 – 4.77
Early infection	1.16	0.62 – 2.19	1.20	0.63 – 2.26
Late infection				
One episode	1.68	1.29 – 2.18	1.68	1.29 – 2.18
Two or more episodes	2.53	1.73 – 3.70	2.55	1.74 – 3.74

Controls with RDS only were matched to cases with BPD according to gestational age by weeks. Conditional logistic regression was performed with adjustment for maternal age in 5-year age groups, birth order, multiple births, and gestational age by additional days in gestational weeks. Early infection=start of symptoms less than 72 hours after birth, Late infections=start of symptoms 72 hours or more after birth.

Pharmacological treatment and ventilator strategies

Treatment with surfactant, postnatal steroids and iNO were more common among cases than controls. Surfactant treatment corresponded to an increased risk of BPD by 58%. Ninety-four percent of those requiring intubation in the delivery room received surfactant. Postnatal steroid treatment corresponded to a 10-fold increased risk of BPD. Of those treated with postnatal steroids, 80% received mechanical ventilation as respiratory support. Treatment with iNO corresponded to almost 7-fold increased risk of BPD. PPHN occurred in 60% of the infants receiving iNO. Almost all infants received CPAP as respiratory support. More cases than controls required mechanical ventilation which yielded a doubled risk of BPD.

Table 9. Exposure to pharmacological treatments and ventilation strategies and the risk of bronchopulmonary dysplasia in infants born <33 gestational weeks, from 2005 to 2010 in Sweden (OR=odds ratio, CI=confidence interval).

Pharmacological treatments	Crude OR	Crude 95%CI	Adjusted OR	Adjusted 95%CI
Surfactant	1.60	1.26 – 2.05	1.58	1.24 – 2.02
Inhaled nitric oxide	6.84	3.24 – 14.41	6.91	3.27 – 14.64
Postnatal steroids				
Overall	10.01	7.23 – 13.84	10.39	7.48 – 14.45
Inhalation	11.48	7.65 – 17.22	12.17	8.04 – 18.41
Injection/per oral	7.82	5.08 – 12.05	7.91	5.12 – 12.22
Ventilation strategies				
nCPAP	1.26	0.51 – 3.09	1.20	0.49 – 2.94
Mechanical ventilation	2.11	1.65 – 2.70	2.12	1.65 – 2.71

Controls with RDS only were matched to cases with BPD according to gestational age by weeks. Conditional logistic regression was performed with adjustment for maternal age in 5-year age groups, birth order, multiple births, and gestational age by additional days in gestational weeks. nCPAP =Nasal Continuous Positive Airway Pressure.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Study design

The RCT is the original epidemiologic study design. The RCT could be thought of as an epidemiologic experiment in which exposures are assigned by randomization.[98] The randomization process intends to prevent confounding.

The observational cohort design could be described as a natural experiment that simulates what would occur in a RCT.[98] In studies I-III we used the *cohort* study design. A cohort study involves the measurements of the occurrence of disease within cohorts.[98] A cohort is defined as any designated group of individuals who are followed over a period of time. Individuals in the cohort have a common characteristic, such as exposure. The cohort study design allows for assessment of many different disease endpoint at the same time.[98] In studies I-II we investigated one exposure (ACS) and its effect of on several disease endpoints. In study III, we explored several prenatal exposures and the effect on one outcome: BPD. In study III, exposure data were available from the whole population of Swedish preterm infants during a 20 year period, allowing us to explore a large number of potential risk factors.

Cohorts could be either *closed* and *opened*.[98] A closed cohort is one with a fixed membership and an open cohort can take new members as time passes. Birth cohorts, such as in studies I-III, are always closed cohorts as all individuals enter at the time point when they are born, despite the fact that the actual date of study start will differ for different individuals.[98]

In study IV, we used the *case-control* study design which aims at achieving the same goals as a cohort study, but more efficiently using sampling.[98] The starting point in case-control studies is the source population, giving rise to the cases. The purpose of the control group, which is sampled from the entire source population, is to determine the relative size of the exposed and unexposed components of the source population. The cardinal requirement of control selection is that the controls are sampled independently of exposure status. In study IV, the source population was preterm infants, live born before 33 gestational weeks and surviving to 28 days or beyond, from year 2005 through 2010 in Sweden. The effect on the outcome, BPD, was investigated for a large number of perinatal exposures.

One disadvantage in case-control studies is that sampling from the source population could lead to inaccurate measure of the exposure distribution and thereby an incorrect risk estimate. Thus, a case-control study may offer less statistical precision in estimating the risk ratio than a cohort study of the same population.[98] To optimize the statistical precision in study IV, we included as cases and controls all infants fulfilling the eligibility criteria and identified in the PNQn register. Thus, the number of cases and control could not be enlarged based on Swedish register data. A way of enlarging the source population in future studies would be to identify cases and controls in similar registers, for example from all the Nordic health registers, merging data from the Nordic population.

Matching was used in study IV. The purpose of matching is to improve efficiency. If the matching factor is a confounder, the stratified analysis will be more efficient in terms of better precision. The cost of matching for a factor is that the effect of that factor can no longer be estimated. As gestational age is a strong and well known risk factor for BPD, we matched controls to cases in study IV by gestational age in weeks at birth. To further increase the precision, gestational age as additional days in each gestational week were adjusted for in the statistical analyses.

Logistic Regression and odds ratio in cohort study design

When comparing an exposed with an unexposed group, the risk ratio (RR) and the odds ratio (OR) are both good indicators of the strength of the association between the exposure and the disease outcome.[97, 99] We decided to present our results based on odds ratios, even though odds ratios are less easy to interpret than risk ratios (or risk differences) [97]. However, when the outcome is rare (prevalence <10%), the odds ratio is a good approximation for the risk ratio.[97-98] This is because the odds of the occurrence of a rare outcome are numerically equivalent to its risk. Therefore, analysis based on odds ratio gives the same results as analyses based on risk ratios. Further, for odds ratios, the conclusions are identical whether we consider our outcome as the occurrence of an event, or the absence of the event.

Logistic regression was used because it allows the analysis of dichotomous or binary outcomes with two mutually exclusive levels and provides the ability to adjust for multiple predictors.[99-100] Thus, logistic regression quantifies the association between a risk factor or treatment and a disease, after adjusting for other variables. This makes logistic regression especially useful for analysis of observational data when adjustment is needed to reduce the potential bias resulting from differences in the groups being compared.[99-100] Logistic regression results are typically presented by odds ratios because these are the natural estimates from the model and attempts to transform these to relative risks can distort the results. [97, 100]Use of standard linear regression for a two-level outcome can produce very unsatisfactory results.[97, 100] Predicted values for some covariate values are likely to be either above the level (usually 1) or below the level of the outcome (usually 0). In addition, the validity of linear regression depends on the variability of the outcome being the same for all values of the predictors. This assumption of constant variability does not match the behavior of a 2-level outcome. Therefore, linear regression is not adequate for such data.[100]

Errors in epidemiological studies

Two types of errors afflict epidemiologic studies; *random* error and *systemati*c error. Random errors could be reduced by increasing the study population but this is not the case for systematic errors. Another term for systematic error is *bias*.[98] *Bias* could be classified into three broad categories; *selection bias*, *information bias*, and *confounding*. In all essentials, the same premise for systemic errors applies for both cohort and case control studies.

Selection bias occurs when participation in a study is associated with the incidence of exposure or outcome studied. In studies I and II, infants born at small hospitals with less than 10 preterm deliveries annually and infants born at hospitals with uncertain routines for antenatal corticosteroid use were excluded. Further, for inclusion into the

cohorts, both studies required that mothers should have been admitted to hospital for more than 48 hours to assure a sufficient time period to allow ACS to have been given and exhibit full effect. Only singleton infants were included based on the fact that multiple birth often is associated with a higher risk of infant morbidity and mortality than singleton infants. In studies III and IV, only infants surviving to 28 or beyond were included. The reason for the 28 days requirement is that, traditionally, infants are diagnosed as having BPD if they have been oxygen dependent for at least 28 days after birth.[48] Further, in study IV, all infants were identified through the PNQn register. Reporting to the PNQn register is not compulsory and the coverage was not nationwide until 2008. Therefore not all preterm infants born during 2005 through 2010 could be identified through the register.

Based on all the reasons for exclusion as outlined above, we may have introduced some selection bias in our studies. However, we do not believe that the selection of study participants have undermined the study results.

Information bias could occur if information on study subjects is erroneous. If the exposure or outcome variable is measured on a categorical scale, erroneous data could lead to an individual being placed in an incorrect category and data being misclassified. There is a risk of misclassification in our all our studies in which data on exposure (studies III-IV) and outcome (studies I-IV) were collected from registers or estimated on an algorithm (studies I-II). However, as information in the registers is prospectively collected by the treating physician at the time of hospitalization, it is unlikely that data suffer from patient non-response bias or recall bias.

In study II, a validation of the exposure algorithm was performed, using medical records. A higher number of individuals were considered exposed based on our predefined method as compared to what was identified in the medical records, which yielded low specificity. When reviewing the medical records we found that information concerning decisions whether to treat or not with ACS was often completely lacking. In addition, written notes of actual ACS administration were surprisingly poor and inconsistently given in different parts of the records. Considering this, one possible explanation for the low specificity is poor documentation i.e. that ACS sometimes was administered without notification in the medical records. Another possibility is that our predefined method actually overestimated exposure status. Nevertheless, if misclassification occurred, we believe that it would be non-differential, i.e. not dependent on the person's disease or exposure status and, if anything, it would produce estimates that are diluted.

Confounding is a systematic error and could be thought of as a mixing of effects.[98] For confounding to occur, confounding the factor must be imbalanced between the compared groups. A confounding factor must be associated with the disease, either as a cause or as a proxy for a cause, but not as an effect of the disease. Further, a confounder must be associated with the exposure, but must not be an effect of the exposure. It should be noticed, that a factor which is an effect of the exposure and an intermediate step in the causal pathway from exposure to disease is not confounder. A causal intermediate is part of the effect that one wishes to study.

Confounding could be handled in the data analysis by stratification and regression models. In all our studies, potential confounding factors were identified before study start and adjusted for in the logistic regression analyses. Stratification is basically cross-tabulation of data on exposure and disease by categories of one or more variables that are potentially confounding variables. Stratified analyses were performed in study II and outcomes were evaluated for infants born late preterm and infants born at term. Other common ways of controlling for confounding in epidemiological studies are restriction and matching.

Potential confounders in studies I and II
In studies I and II, exposure status was estimated at the hospital level. As a true confounder should be related to both exposure and outcome, we adjusted for confounding factors that could interfere with both hospital of birth and outcome. Therefore, year of birth, hospital level and gestational age at birth (study I) and pregnancy duration (gestational age) at first hospital admittance by second degree polynomial (study II) were identified as potential confounders and adjusted for in the statistical analyses. Year of birth adjusted for differences in neonatal care during different years. Hospital level was included as there is evidence that the risk of neonatal mortality and morbidity is higher in preterm infants born at hospitals providing basic care as compared to infants born at hospitals with specialized neonatal care.[2, 101] As exposure was estimated on hospital level, we did not adjust for individual factors, such as gestational diabetes or maternal infections during pregnancy, which could influence whether a specific individual was given ACS or not.

Potential confounders in studies III and IV
Maternal age and birth order were adjusted for in both studies III and IV. Maternal age was included as confounder since increasing maternal age is associated with an increased risk of adverse pregnancy outcome in general. In addition, birth order is correlated to the age of the mother, as women giving birth to their second or third child, are generally older than mothers giving birth to their first child.

In study III, we further adjusted for hospital level, smoking habits, year of birth and if the mother was born in a Nordic country. Year of birth adjusted for differences in neonatal care during different years. Whether the mother was born in a Nordic country or not adjusted for increased risk of adverse pregnancy outcome related to ethnic background. In addition, we adjusted for birth weight by gestational age (i.e. small for gestational age [SGA], large for gestational age [LGA]) when testing the association between BPD and preeclampsia related disorders, diabetes mellitus and gestational diabetes. The rationale for this is that preeclampsia is associated with decreased fetal growth whereas diabetes mellitus/gestational diabetes is associated with increased fetal growth.

In study IV, we also adjusted for multiple births and gestational age as additional days in each gestational week. Multiple births were adjusted for because it is often associated with a higher risk of infant morbidity and mortality. Gestational age was adjusted for as controls were matched to cases according to gestational age at birth and there is a risk of introducing bias if adjustment for the matching factor is not done in the logistic regression analysis.

Internal and external validity and precision

Internal validity is related to the accuracy of the study results. If the study result is trustworthy and close to the true value in the studied population, the internal validity is high. Bias and confounding have negative effects on internal validity. In all studies, effort were made to identifying potential confounders which to be adjusted for in the statistical analysis. Further, by using prospectively collected register data, recall bias and patient non-response bias are minimized, which increases the internal validity of the study results.

External validity is related to the generalizability of the study results. If the study result could be extrapolated to the results of a larger population, the external validity is high. External validity is sensitive to selection bias. In our studies we included the whole Swedish population of infants that fulfilled the study criteria, during the actual study period and the study results could be generalized to populations of preterm infant born in an era of modern neonatal care.

Precision is related to random errors and could be reduced by increasing the number of subject in study population.

Effect modification

Interaction can be statistical or biologic, causal (synergism, antagonism). There is substantial confusion surrounding the evaluation of interaction, much of which stems from the fact that the term is used differently in statistics and epidemiology.[98] In statistics, the term interaction is used to refer to departure from the underlying form of a statistical model. Biologic interaction between two potential causes occurs whenever the effect of one is dependent on the presence of the other. When interaction is present, the association between the risk factor and outcome variable is not constant over different levels of the interacting covariate. That is, the interacting covariate modifies the effect of the risk factor. Therefore, any statement about the risk factor or exposure effect will have to specify the covariate level.

Summary

Assessing the effects of exposure (drug treatment or diseases) in a clinical setting is more complex than in the context of a RCT. Our design (observational cohort studies and case-control study) does not allow us to firmly conclude that it is exclusively the exposure in itself that caused the association with the outcomes. We tried, however, to compensate for all potential shortcomings by ascertaining both exposure and outcome and adjusting for identified potential confounders. Nevertheless, the risk of misclassification of exposure or outcome could not be disregarded. However, if misclassification is present it is more likely to be non-differential, which would dilute the association between exposure and outcome. In addition, the result was in line with the result from previous RCTs.[7, 32] As all these studies emerge from a large nationwide population, the results could be generalized to infants born in industrial countries in an era of modern neonatal care.

6.2 DISCUSSION OF THE RESULTS

The overall aim of this thesis was to evaluate the comprehensive health consequences of the widespread routine treatment with ACS to women with imminent preterm delivery and to increase the knowledge of risk factors implicated in the development of BPD.

The results from studies I and II confirm that treatment with ACS reduces the occurrence of RDS in the population of infants that actually receive the treatment in real life, with no apparent increased risk for long-term adverse effect. Not only infants born before gestational week 34 will benefit from the treatment, also exposed infants born at a later gestational age had a reduced risk of RDS. This indicates a prolonged beneficial effect of previous ACS treatment. Study III found that preeclampsia was a strong risk factor for BPD and that gestational diabetes and diabetes mellitus decreased the risk. The overall conclusion from study IV is that infants who later on develop BPD are more severely ill already at birth. Infants who developed BPD had more often been exposed to long duration of PPROM, they were more often SGA and had lower Apgar scores and required resuscitation interventions in the delivery room at a higher rate as compared to infants with RDS but not developing BPD. These findings indicate that infants subsequently developing BPD had been exposed to factors causing lung injury and triggering inflammation already during fetal life.

6.2.1 Studies I and II

The magnitude of effect on RDS in our observational study was not as large as reported from reviews of RCT.[7, 32] However, assessing the effect of a single procedure in a full clinical setting is more complex than using the experimental design of an RCT. Other postnatal treatments that could not be controlled for in the study could have compensated for lack of prenatal treatment.

Studies I and II contribute to the knowledge of long-term effects of ACS, data which could not have been sufficiently achieved from RCTs. None of our studies could detect any apparent adverse long-term neurological effect and no increased risk of neonatal death was seen. The only significant adverse finding was an increased risk of low Apgar score in exposed infants born term. Low Apgar score could be related to long-term neurological effects such as epilepsy or cerebral palsy or impact on more subtle neurosensory outcomes, such as school performance and intellectual capacity. We included nearly 12 000 infants in study II and found no increased risk of epilepsy or cerebral palsy. Whether the finding of low Apgar score had any clinical significance on school performance cannot be concluded as no such endpoints were possible to evaluate.

To assess rare outcomes, large populations are needed to be followed for a long period of time. Major limitations of previous observational studies have been the small number of included infants.[7, 102] By using the observational cohort design and extracting data from national registers we managed to include the majority of Swedish preterm newborns during a 20 year period and follow the children during their first decade in life. The lack of significant long-term adverse effects adds valuable information to the knowledge of the total health consequences of ACS treatment.

When comparing the numbers of infants identified as preterm, late preterm and term in both studies, the proportion of false imminent preterm deliveries and the number of women in whom pregnancy continued to term was surprising as it was larger than the proportion of preterm births. A previous publication reported that approximately 25% of women presenting with imminent preterm delivery will not deliver until after one week or more.[103] However, our data indicate that the proportion of false imminent delivery might be even larger. As both studies were observational studies, the gestational age distribution in the study cohorts mirrored the actual age distributions in real life. In study I, 7 827 infants were born preterm which should be compared with the 11 873 infants identified in study II who were born to women admitted to hospital for imminent preterm delivery but who gave birth late preterm or term. This illustrates that for every suspected preterm delivery, giving rise to an infant born before gestational week 34, there will be another one or two infants born at a later gestational age.

The clinical judgment whether preterm delivery is inevitable or not is difficult and impacts on the decision whether to give ACS or not. In this aspect, our studies cannot contribute to any new knowledge facilitating the decision on which women should receive treatment with ACS or not. Future studies should focus on identifying those women who actually will proceed into preterm delivery among all women presenting with perceived preterm labor. As a large number of these women will not give birth within a short period of time, the question of repeated ACS treatment will arise. As there is uncertainty over the benefits and long term adverse effects of repeated courses of ACS given 7 days after the previous course, future research should focus on evaluating repeated course of ACS.

6.2.2 Study III

In line with other publications, the results in study III show that preeclampsia was a strong prenatal risk factor for BPD development.[104] The effect was apparent in all infants born preterm and in extremely preterm infants. The pathophysiology of preeclampsia is not completely understood but it is primarily a vascular disease, induced by an imbalance in angiogenetic factors.[105-108] The increased risk for BPD could be related to inflammatory mechanism but an impaired angiogenesis may also play a significant role.[23, 109] Although not statistically significant, we found a doubled risk of BPD in extremely preterm infants, born to women with endometriosis. This finding could also be associated with an impaired angiogenesis. Therefore, further studies investigating associations between BPD and diseases in which angiogenetic factors may play a role are warranted.

In contrast to the increased risk of BPD associated with preeclampsia, a reduced risk associated with maternal diabetes mellitus and gestational diabetes was found. The mechanism behind such a protective effect is unclear and we tried to explore whether the effect was related to the disorder itself, its treatment or other factors related to the disorder. Maternal diabetes is commonly associated with an increased risk of accelerated intrauterine growth and large for gestational age infants, whereas preeclampsia is associated with an increased risk of growth restriction.[110-111] To explore

the impact of birth weight, we performed additional analyses adjusting for birth weight by gestational age (SGA/LGA), but found only minor effects on the risk estimates. To further adhere to the assumption of an impaired angiogenesis as part of the causal pathway of BPD, one could hypothesize how insulin treatment during pregnancy may act as a protective factor. Insulin, being a growth hormone, may promote angiogenesis and facilitate vascular pulmonary development. To investigate the role of insulin treatment during pregnancy, we performed additional analyses, separately assessing infants born to gestational diabetic mothers with and without insulin, but found no protective effect of insulin treatment on the risk of BPD. Considering the diabetic disorder itself, one could speculate that a poor metabolic control during pregnancy causing infant hypoglycaemia in the postnatal period might initiate infant stress reactions and increase endogenous corticosteroid levels, thus, promoting lung maturation in the newborn infant. However, we did not succeed in clarifying the mechanism and the protective effect of diabetes remains puzzling. To our knowledge, no published study has specifically investigated this association but the result is in line with the higher rates of BPD in non-diabetic mothers previously reported by Bental et al.[112] Additional studies are needed to further investigate the association.

The role of chorioamnionitis in BPD development is unclear and the results from previous studies are ambiguous. Our findings of an increased risk in all infants born before gestational week 37, but not in infants born extremely preterm, do not clarify whether chorioamnionitis is a true risk factor or not.

We also found an association between lower urinary tract infections and risk of BPD in infants born before 37 gestational weeks in general, but not specifically in infants born extremely preterm. According to published literature, lower urinary tract infection does not seem to be related to adverse pregnancy outcome.[113] Additional studies are needed to confirm or reject our finding of an increased risk of BPD associated with lower urinary tract infections.

Antibiotics were associated with a decreased risk of BPD and it appears that maternal antibiotic treatment might have beneficial effect on BPD. One possible explanation is that drug treatment during pregnancy could be a proxy for closer pregnancy follow-up, which could influence the outcome of pregnancy.

One of the main objectives of this thesis was to investigate whether maternal inflammatory diseases affected the risk of BPD development. No apparent association was found between BPD and maternal chronic inflammatory diseases or use of anti-inflammatory drugs. Thus, maternal inflammatory diseases in general do not seem to increase the risk for BPD. However, a weakness in our study was that we did not have access to information on maternal disease flare or remission. We could therefore not estimate the magnitude of maternal systemic inflammation on exposed infants. As development of BPD is multi-factorial including several causal factors for which duration and magnitude of exposure may play significant roles, our results do not allow conclusions regarding risks in infants exposed to high level of maternal systemic inflammation, such as that occurring in relation to a flare.

6.2.3 Study IV

A relevant clinical question is how to identify those infants at higher risk of BPD, among those preterm infants presenting with respiratory problems. An early identification of infants at risk, requiring more support and intensive treatment, is likely to be beneficial for the infants and an advantage for planning strategies at neonatal intensive care units.[114]

Study IV compared infants with BPD with infants with RDS only, as we found such comparison of more relevance for the clinical situation than comparison with an unselected group of infants as done in most previous studies.[61, 63, 115] Study III also investigated risk factors for BPD development but a major difference between these two studies is the comparison group, which in study III was an unselected group of preterm infants. The importance of a specific risk factor could differ in these populations. To our knowledge no previous published study has focused on the specific comparison between infants with BPD and infants with RDS only.

Growth related risk factors
SGA doubled the risk of BPD. An SGA neonate may be constitutionally small but more often SGA is the consequence of utero-placental insufficiency.[116] Utero-placental insufficiency is associated with inflammatory or vascular disorders, for example preeclampsia.[117] In study IV, we tested whether preeclampsia was the underlying cause behind the association between SGA and BPD and found an interaction. Somewhat surprising, the effect of SGA on BPD development was higher among growth restricted infants due to other causes than preeclampsia as compared to the effect of SGA related to preeclampsia. Preeclampsia is a temporary disease, usually starting late in gestation. Both mother and infant will usually recover from symptoms shortly after delivery.[118] The finding that other factors than preeclampsia had a greater impact on BPD in relation to SGA, may suggest that other unknown factors causing irreversible growth restriction of the lungs may play a role in the development of BPD. Further, no association with BPD was found with other growth related factors, such as gestational diabetes and maternal smoking. In study III, we also investigated the effect of preeclampsia on BPD and found that preeclampsia was a strong prenatal risk factor in all preterm infants and infants born extremely preterm. One reason for diversity in result is the difference in comparison group. The importance of preeclampsia as a risk factor could differ in these populations.

Inflammatory risk factors
Duration of PPROM exceeding one week tripled the risk of BPD. PPROM could be seen as a proxy for chorioamnionitis as bacterial infection is the main cause for membrane rupture. Moreover, PROM has been shown to correlate to clinical and histological chorioamnionitis.[119-120] Whether chorioamnionitis is a risk factor for BPD has not been established but in preterm infants exposure to inflammation related to chorioamnionitis has been proposed as a risk factor since intra-amniotic endotoxin exposure can disrupt alveolar development.[121] The effect of chorioamnionitis was also investigated in study III with a somewhat indistinct result, showing an increased risk in infants born before gestational week 37 in general, but not specifically in extremely

preterm infants. The finding in study IV supports the association between chorioamnionitis and BPD.

Severe postnatal infections have been shown to increase the risk of BPD, which is supported by our finding of higher risk of BPD with more episodes of infection.[122] However, there is a complex interplay between several of the investigated risk factors. The risk of severe infection increases with longer duration of ventilator support and infants with severe infections have an increased risk of a reopening of PDA and PDA increases the risk of BPD.[122] Nevertheless, our finding underscores the importance of strategies to reduce infections in preterm infants.

Interventions in relation to delivery
Infants who subsequently develop BPD had lower Apgar score and higher needs of resuscitation in the delivery room as compared to infants with RDS. This strengthens the assumption of prenatal factors causing lung injury as part in the causal pathway of BPD development. However, resuscitation interventions per se may also cause lung injury and it is difficult to establish whether these interventions were required because of the lung injury or actually caused it.[123-124]

Associated co-morbidities
A substantial difference in the occurrence of co-morbidity was noticed as infants with BPD more often suffered from other conditions. Presumably, several of the assessed co-morbidities are not risk factors per se, but rather co-factors and it has not been possible to separate true risk factors from factors that might co-exist with BPD.

We found a strong association between PDA and BPD with the highest risk in infants requiring surgical ligation. Fluid overloads and surgical ligation of PDA have been suggested as causal factors of BPD.[45-46, 50] Although it is difficult to determine if the increased risk is due to the ligation itself, we propose that the association is related to other factors such as prolonged exposure to PDA which might coexist in infants who require ligation. In infants with PPHN, we found a 5-fold increased risk of BPD and both PIE and pneumothorax more than doubled the risk of BPD. However, the majority of infants with PIE and pneumothorax received mechanical ventilation and the observed association with PIE and pneumothorax might be a consequence of the respiratory support.

Pharmacological treatment
ACS is a clinically relevant modulator of lung maturation which reduces the incidence of neonatal death and RDS in preterm birth. It has been suggested that ACS may increase the risk of BPD, but our results do not support such an association. The increased risk associated with *postnatal* steroid treatment probably mirrors the more severe respiratory morbidity seen in infants with BPD.

In summary, the results from study IV show that both pre- and postnatal factors related to growth restriction and inflammation were associated with an increased risk of BPD. Presumably, several of the assessed postnatal factors are not risk factors per se, but rather co-factors. An infant presenting with respiratory problems and born after

PPROM or as SGA with low Apgar score, and who requires resuscitation in the delivery room should alert the suspicion of subsequent development of BPD.

6.2.4 Mode of delivery in studies I-IV

Cesarean section increases the risk for postnatal respiratory morbidity in general and has been shown to be a risk factor for RDS.[25-26, 125]

Studies I-II evaluated the effect of ACS exposure on both short and long term outcomes. Information on mode of delivery (vaginal or cesarean section) was not available in these studies. Further, we did not consider mode of delivery as related to whether an infant was classified as exposed to ACS or not at hospital level. Therefore mode of delivery was not included as a potential confounder in studies I and II.

In study III data on mode of delivery was available and presented as maternal and infant characteristics in Table 1. Cesarean section was far more common in infants with BPD. The primary objective of study III was to evaluate *prenatal inflammatory* risk factors and cesarean section did not qualify for that criteria. Therefore, cesarean section was not evaluated as a risk factor. To test whether mode of delivery modified the effect of a risk factor we carried out additional analyses including cesarean delivery as effect modifier. Only minor changes on the risk estimates were seen.

Table 10. Exposure to cesarean section and the occurrence of bronchopulmonary dysplasia (BPD) in infants born before 37 gestational weeks included in study III presented in a 2*2 table.

Cesarean section	BPD Yes	BPD No
Yes	64.1%	34.6%
No	35.9%	65.4%

In study IV, the effect of mode of delivery was evaluated in infants born before 27 gestational weeks and in infants born from gestational week 27 to gestational week 32. In both age groups an increased risk estimate was found among those delivered by cesarean section but none of the risk estimates was statistically significant.

Table 11. Cesarean section and the risk of bronchopulmonary dysplasia by gestational age in infants included in study IV (OR=odds ratio, CI=confidence interval).

Gestational age at birth (weeks)	Adjusted OR	Adjusted 95%CI
< 27	1.27	0.89 – 1.82
≥ 27	1.38	0.97 – 1.95

6.2.5 Gender distribution in studies I-IV

There were more male than female infants in the study population of all our studies. Boys have higher perinatal mortality and morbidity in general and higher risk of preterm birth. Both RDS and BPD and other respiratory diseases are more common in boys than in girls.[126-127]

The understanding of whether sex impacts on the risk of BPD development may add valuable information of which infant that is at risk and will require more intensive care

interventions. However, sex of the infant is not a variable that could be prevented in order to reduce the risk of BPD.

Table 12. Distribution of infant gender among infants exposed or unexposed to antenatal corticosteroids (ACS) in study I and II.

Sex	Exposed to ACS	Unexposed to ACS
Study I		
Female	45.2%	43.8%
Male	54.8%	56.2%
Study II		
Female	45.9%	44.6%
Male	54.1%	55.4%

In study I, we performed analyses stratified according to gender. With the exception of epilepsy, there was no difference in the short and long term risk between male and female infants. Both male and female infants had a reduced risk of RDS after ACS exposure and no association with BPD was seen. In study II, the BPD events were too few for meaningful analysis.

Table 13. Exposure to antenatal corticosteroids and the risk of respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD) by gender in study I (OR=odds ratio, CI=confidence interval).

	Male		Female	
	Adjusted OR	Adjusted 95%CI	Adjusted OR	Adjusted 95%CI
RDS	0.76	0.64 – 0.90	0.88	0.72 – 1.08
BPD	0.78	0.52 – 1.17	1.04	0.61 – 1.78

The population in studies III and IV also consisted of more boys than girls and BPD was more common among boys. A reason for the higher number of boys in these studies is that preterm birth and neonatal morbidity is more common in boys than girls.

Table 14. Distribution of infant genders among infants with and without bronchopulmonary dysplasia (BPD) in study III and IV.

Sex	BPD Yes	BPD No
Study III		*
Female	37.0%	45.9%
Male	63.0%	54.1%
Study IV		**
Female	38.1%	44.3%
Male	61.9%	55.7%

* All infants born before 37 gestational weeks

** Infants diagnosed with RDS and born before 33 gestational weeks.

7 CONCLUSIONS

- The prophylactic use of ACS in the clinical setting in modern neonatal care has a similar beneficial magnitude on RDS as previously shown in RCTs and treatment is not afflicted with any apparent increased risk of negative long-term neurosensory outcomes such as epilepsy or CP.

- Infants born late preterm have prolonged beneficial effect of earlier corticosteroid exposure with reduced risk of RDS. For infants born at term, the total treatment effect was less beneficial as they had increased risk of low Apgar score.

- Preeclampsia is a strong risk factor for development of BPD in preterm infants. Maternal diabetes mellitus and gestational diabetes reduced the risk of BPD, implying that an impaired angiogenesis could be added to the possible etiological factors that contribute to BPD development.

- Both pre- and postnatal factors related to growth restriction and inflammation are associated with an increased risk of BPD. An infant presenting with respiratory problems and born after PPROM or as SGA with low Apgar score and resuscitated in the delivery room should alert the suspicion of subsequent development of BPD.

8 FUTURE PERSPECTIVE

There is an uncertainty over the benefits and long term adverse effects of repeated courses of ACS given 7 days after the previous course. Future research should focus on evaluating repeated course of ACS before this could be routinely recommended.

Future studies should focus on identifying those factors responsible for intrauterine growth restriction and associated with development of BPD.

Further investigations concerning associations between diseases in which angiogenic factors play a role and BPD are warranted.

The susceptibility of some infants for BPD development may not be only environmental, but also genetic. Studies on genetic association may add further knowledge to the etiology of BPD development.

Major questions remain concerning growth of the lungs through childhood and adult age in those diagnosed with BPD. Research should focus on long term follow-up of infants with BPD.

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