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LUNG FUNCTION MEASURED WITH SPECT IN INFANTS AND CHILDREN WITH BRONCHOPULMONARY DYSPLASIA -CORRELATION WITH RESPIRATORY MANAGEMENT AND CLINICAL GRADING

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Lung function measured with SPECT in infants and children with Bronchopulmonary Dysplasia

-Correlation with respiratory management and clinical grading

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Denna avhandling är tillägnad mina döttrar Clara, Ebba och Felicia, ni är mina stjärnor

ABSTRACT

Chronic lung disease, bronchopulmonary dysplasia (BPD) is a common and severe complication among extremely preterm born infants and infants with lung hypoplasia. Clinical grading of BPD is performed at 36 weeks postmenstrual age (or 56 days of age in moderately preterm infants) based on the need for supplemental oxygen. Oxygen requirement is a poor predictor for lung function in childhood and at adult age. We aimed to evaluate a lung function measurement for infants and children with BPD to complement clinical grading, evaluate the impact of respiratory management and standardize follow-up. No functional imaging of the lung in neonatal patients has been reported earlier. Single photon emission computed tomography (SPECT) is a technique that gives a functional imaging at a regional level within the lung. Previously the technique has not been available to use in non-cooperating patients that are small in body size. Recent technical advances and the development of a system for inhalation by our research group opened up the possibility of applying SPECT in neonatal patients. Our aim was to see if three different groups of patients, extremely/very preterm born infants, children with BPD or infants with congenital diaphragmatic hernia and lung hypoplasia, had lung function impairment with ventilation perfusion abnormalities within the lung.

In paper I and IV we compare ventilation (V) perfusion (Q) matching with clinical severity grading, clinical characteristics and respiratory support during the first week of life (paper IV) and for the whole stay in the neonatal unit (paper I). V/Q abnormalities were correlated with BPD severity grade. Increasing days with mechanical ventilation (MV) were negatively correlated with lung function measured by SPECT. Higher values of carbon dioxide on the first day of life and during the first week were associated with better V/Q matching. High mean airway pressure (MAP) day 1 of life, a large decrease in MAP and early weaning of mechanical ventilation were also associated with better V/Q matching. High MAP and oxygen demand at day seven of life were associated with an increased severity grade of BPD. Additionally we found that one third of the patients graded as Mild or Moderate BPD had widespread V/Q abnormalities.

In paper II we studied patients with lung hypoplasia and congenital diaphragmatic hernia. In this group, V/Q abnormalities were also associated with increasing days with MV but also increasing days with extracorporeal membrane oxygenation and persistence of pulmonary hypertension.

In a follow-up study of 10-year-old children with BPD born preterm (paper III), we found less V/Q abnormalities than in the preterm infants but lower values of lung volume with good V/Q matching than expected in lung healthy children at that age.

SPECT can be used as a measurement of lung function and evaluation of treatment in neonatal patients, infants and children and as a compliment to clinical grading and for follow-up of lung function in survivors with BPD. Ventilation perfusion matching abnormalities are common among patients with BPD both in the newborn period and at school age.

LIST OF SCIENTIFIC PAPERS

I. **Bronchopulmonary Dysplasia: Clinical Grading in Relation to Ventilation/Perfusion Mismatch Measured by Single Photon Emission Computed Tomography**
Kjellberg M, Björkman B, Rohdin M, Sanchez-Crespo A, Jónsson B.
Pediatric Pulmonology, 2013: 1206-1213, DOI: 10.1002/ppul.22751

II. **Postoperative regional distribution of pulmonary ventilation and perfusion in infants with congenital diaphragmatic hernia**
Björkman K, Kjellberg M, Bergström S E, Jónsson B, Lindahl S, Radell P, Rohdin M, Sanchez-Crespo A
Journal of Pediatric Surgery, 2011:2047-2053, doi: 10.1016/j.jpedsurg.2011.06.042

III. **Ten-year-old children with a history of bronchopulmonary dysplasia have regional abnormalities in ventilation perfusion matching**
Kjellberg M, Sanchez-Crespo A, Jónsson B
Pediatric Pulmonology, 2019;1-8. https//doi.org/10.1002/ppul.24273

IV. **Respiratory Management the First Week of Life Affects BPD Grading and Ventilation Perfusion Matching at Term Age**
Kjellberg M, Sanchez-Crespo A, Jónsson B
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

BPD	Bronchopulmonary dysplasia
c	Contralateral
CDH	Congenital diaphragmatic hernia
CPAP	Continuous positive airway pressure
CT	Computed tomography
ECMO	Extracorporeal membrane oxygenation
FiO2	Fraction inspired oxygen
GA	Gestational age
i	Ipsilateral
IPPS	Intermittent positive pressure support
MAP	Mean airway pressure
MIGET	Multiple inert gas elimination technique
MRI	Magnetic resonance imaging
MV	Mechanical ventilation
NDI	Neurodevelopmental impairment
OI	Oxygenation index
pCO2	Partial pressure of carbon dioxide in blood
PDA	Patent ductus arteriosus
PEEP	Positive end-expiratory pressure
PMA	Postmenstrual age
PFT	Pulmonary function tests
PHTN/PPHN	Persistent pulmonary hypertension of the newborn
PULLM study	PrematUre follow up Lung function Mannitol and Metacholine
Q	Perfusion
RDS	Respiratory distress syndrome
SGA	Small for gestational age
SPECT	Single photon emission computed tomography
V	Ventilation
V/Q	Ventilation/ Perfusion ratio
VLBW	Very low birth weight
99mTc-MAA	Technetium 99m labeled macro aggregate albumin

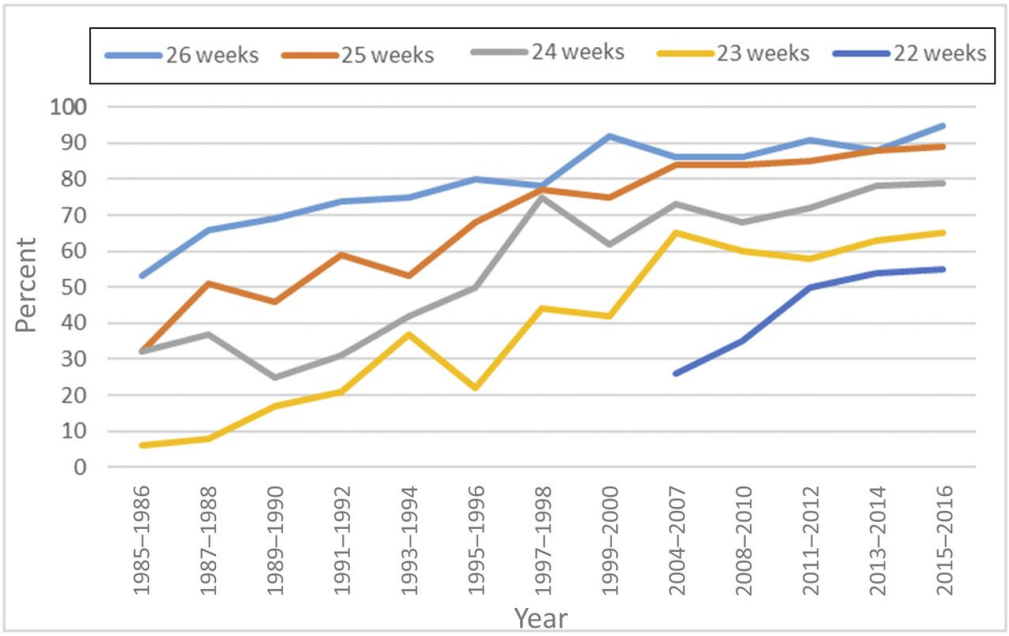
1. BACKGROUND

1.1 SURVIVAL AT THE LIMIT OF VIABILITY

Survival of infants born at the limits of viability has steadily increased in the last decades. Sweden has been in the front-line of management of these infants for many years. About 30 years ago only 25% of infants born at 24 weeks gestational age survived while this figure today is approaching 80% [1]. In infants born today before 25 week gestational age about 75% will require supplemental oxygen at 36 weeks postmenstrual age [2]. After improvement in preoperative management for infants with congenital diaphragmatic hernia the survival rate has increased to 85% in Stockholm compared to 69% internationally [3]. Also patients with severe lung hypoplasia can now survive. What impact extreme preterm birth and severe lung hypoplasia have on future pulmonary function in these children is unknown.

Figure 1. Neonatal survival rate 1985-2016 in Sweden [1]

Neonatal survival of extremely preterm, live-born infants at 22 to 26 completed weeks' gestation in Sweden between 1985 and 2016 by gestational age and year of birth.



Magnus Domellöf, and Baldvin Jonsson Pediatrics 2018;142:S533-S538

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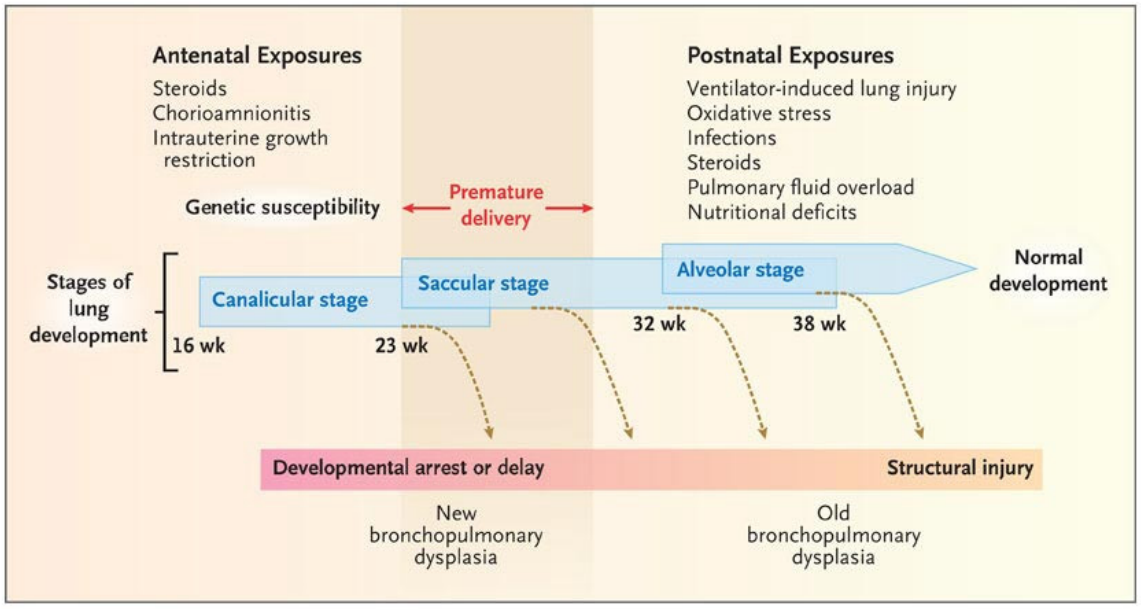
 Neonatal survival 1985 and 2016 in Sweden of live-born infants at a gestational age of 22 to 26 completed weeks.

1.2 NORMAL DEVELOPMENT OF THE LUNG

Alveolarization of the lung starts at around 32 weeks gestational age and continues until the age of two years. There is some evidence that it can continue to some extent even after this period [4,5]. Extremely preterm infants are born before alveolarization has started at a time point when the lung is very vulnerable to injury. Prenatal and postnatal exposures to potentially harmful factors can lead to an arrested development or structural damage within the lung.

Figure 2. Stages of lung development, risk factors, and types of lung injury [6]

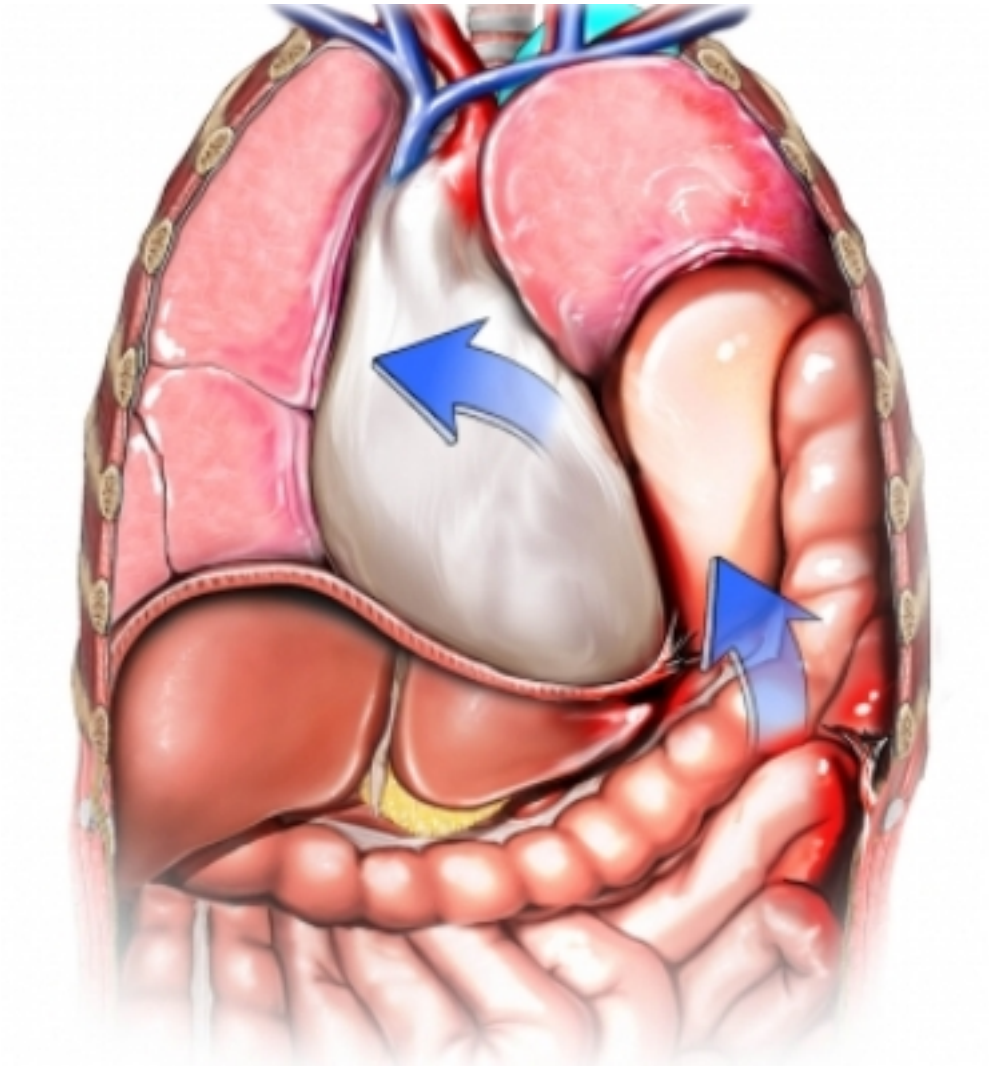


In infants with congenital diaphragmatic hernia the mechanism leading to a reduced lung function is different. Congenital diaphragmatic hernia (CDH) is a developmental defect occurring in 2,3 per 10 000 births with a survival rate of about 70% the first week of life [7]. The diaphragmatic defect is usually posterolateral (Bochdalek hernia), but may be anterior retrosternal or parasternal (Morgagni hernia), or rarely central.

CDH is associated with lung hypoplasia on the same side as the hernia (ipsilateral) but the lung on the other side (contralateral) can also be affected. Bronchial and pulmonary arterial branching is reduced in utero due to compression from the hernia on the ipsilateral lung and if there is a mediastinal shift also the contralateral lung. Reduced arterial branching results in muscular hyperplasia of the pulmonary arterial tree, which contributes to increased risk of persistent pulmonary hypertension of the newborn [8]. The pulmonary vascular bed in CDH patients is not only reduced in size but also responds abnormally to vasodilators with an increased sensitivity in all pulmonary vessels [9]. Severe pulmonary hypoplasia and persistence of pulmonary hypertension are the biggest risk factors for an adverse outcome [10,11]. McGoon index is the combined diameter of the right and left pulmonary arteries compared to the

descending aorta. In infants with congenital diaphragmatic hernia a modified McGoon index has been used. A cutoff value of 1.25-1.3 predicted mortality with 73-85% sensitivity [12,13].

Figure 3. Left sided congenital diaphragmatic hernia compressing ipsilateral and contralateral lung



1.3 BRONCHOPULMONARY DYSPLASIA

Bronchopulmonary dysplasia (BPD) remains a common and sometimes severe complication in extremely preterm born children and infants with underlying pulmonary abnormalities [14,15]. Today with the progress in neonatal care for extremely preterm infants, including prenatal steroids, surfactant therapy and gentler mechanical ventilation and for moderately preterm infants treatment with inhaled nitric oxide and extra corporal membrane oxygenation, increasing numbers of sicker and more preterm infants survive. How we treat these children in the beginning can have an impact on lung function at full term age, during childhood and the rest of their lives.

1.3.1 Incidence

In Sweden all infants born at 23 weeks gestational age will develop BPD. [16] In infants born ≤ 25 weeks gestational age and 25-27 weeks gestational age 75% and 45% respectively will develop moderate or severe BPD [17]. Several studies though, report airway abnormalities also in preterm born infant without BPD compared to term born controls [18,19].

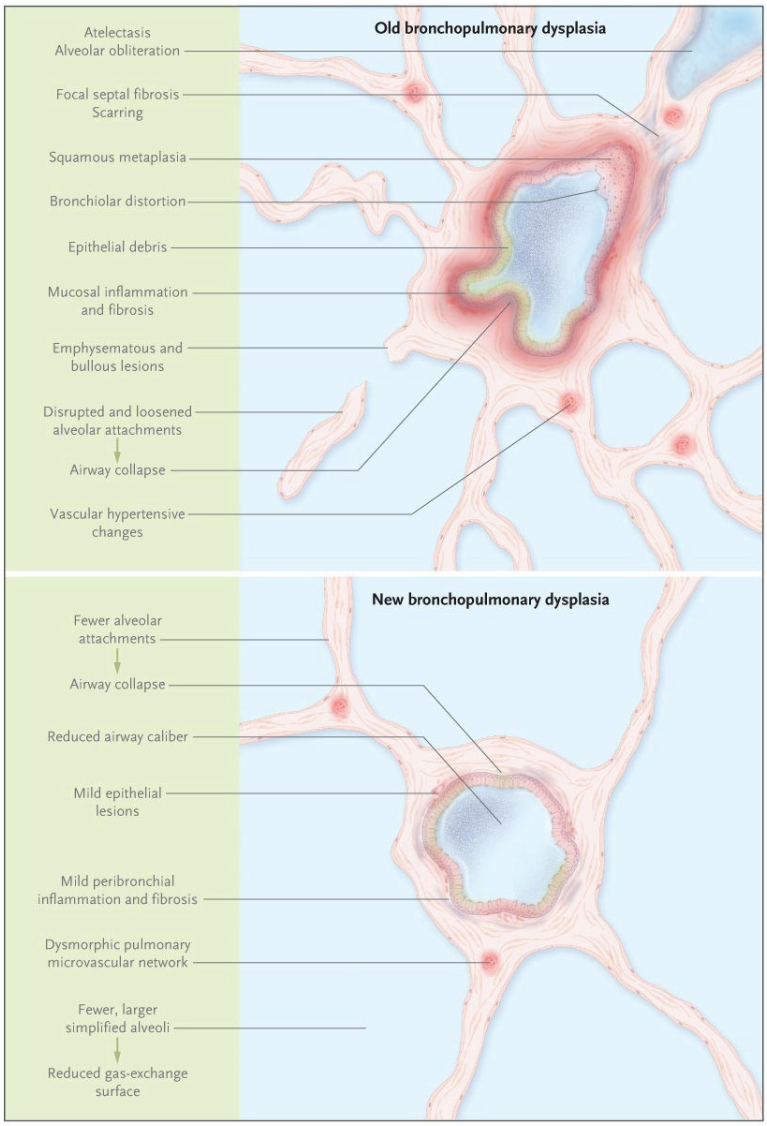
In infants with congenital diaphragmatic hernia the incidence of BPD is about 30%-49% in surviving infants [20,21].

1.3.2 Definition of BPD

When BPD was first described by Northway et al in 1967 the severe histopathological and radiographic changes formed the base for diagnosis [22]. He reported atelectasis, emphysema, fibrosis, smooth muscle hypertrophy and widespread vascular changes within the lung in 19 non-surviving moderately preterm infants treated with mechanical ventilation and oxygen for respiratory distress syndrome (RDS). He coined the term bronchopulmonary dysplasia and described 4 radiographic stages of BPD in these infants and in 13 infants who survived. The mean gestational age for survivors and non-survivors were 34 and 31 weeks respectively. Toce *et al* suggested a combined clinical and radiographic scoring system to enable comparison between different centers. The scoring should be performed at 21 days of age in infants at risk of developing BPD [23]. The radiographic scoring system was a modification of the one suggested by Edwards [24]. Clinical factors evaluated included respiratory symptoms like retractions, weight gain, oxygen demand and pCO2. The patients in this study had a mean gestational age of 30.9 and 32.6 weeks.

In 1988 Shennan *et al* proposed that oxygen need at the time of discharge, around 36 weeks postmenstrual age, was a better marker for severity of disease than an chest X-ray [25]. With improvement in neonatal care smaller and more preterm infants survived and developed respiratory impairment but without the typical radiographic and histopathological changes. Instead alveolarization and vascular development was arrested or delayed resulting in a reduced alveolar surface area [26]. This is now described as New BPD [27]. "Old" BPD is characterized by damaged lung parenchyma where atelectasis, fibrosis, inflammation and emphysema dominate the picture, while in "new" BPD arrested alveolar development and dysmorphic vascularization are the main features leading to fewer and larges alveoli and a reduced alveolar area. Newer research indicates the presence of precapillary arteriovenous anastomotic vessels in the lungs of diseased infants with severe BPD. These vessels could contribute to intrapulmonary shunt and hypoxemia in neonates with BPD [28].

Figure 4: Old versus new BPD. [6] Type of parenchymal damage.

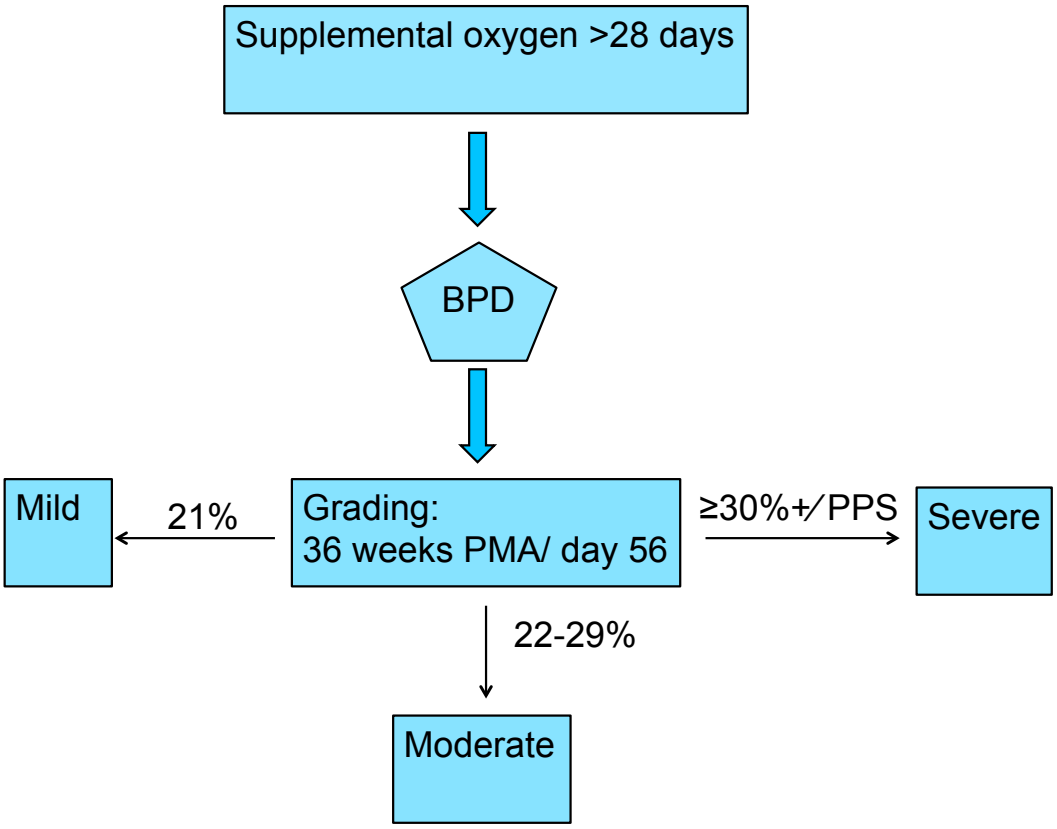


A further modified definition of BPD came in 2001 from a workshop at the National Institute of Health. The diagnosis of BPD was set if there was a sustained need for supplemental oxygen for 28 days or more and the severity grading was performed at 36 weeks postmenstrual age for infants born before 32 weeks gestational age (GA) and otherwise at 56 days of age [29].

1.3.3 Severity grading

The severity grading was based on need for supplemental oxygen and/or positive pressure support as follows;

Figure 5. Diagnosis and clinical grading of BPD [29]



PPS: Positive pressure support, PMA: Post menstrual age
Mild: No need for supplemental oxygen or positive pressure respiratory support to maintain an adequate saturation
Moderate: Supplemental oxygen 22-30% but no need for positive pressure respiratory support. Oxygen reduction test can be performed.
Severe: More than 30% oxygen and/or need for positive pressure respiratory support

This definition has been criticized since use of supplemental oxygen differs between hospitals and individual families after discharge. To get a more standardized definition for research, follow up and comparison between centers Walsh developed the "Oxygen reduction test" [30,31]. In summary, this test, which is performed at 36 weeks postmenstrual age in patients, graded as Moderate BPD by the NIH definition, are defined as non-BPD if they maintain saturations above 90% in room air for 60 minutes and defined as BPD if they fail to do so. This definition reduced the incidence of BPD since Mild BPD and some infants in the group of Moderate BPD according to the NIH definition are now defined as non-BPD. This simplification may not be an optimal predictor for long-term pulmonary outcome.

The most common definition in use today and the definition used in my research is the NIH definition from 2001. However, the predictive value of grading according to this definition has been questioned [32]. A concern is that the clinical oxygen and/or positive pressure criteria at 36 weeks may not identify important pulmonary abnormalities that can lead to a reduced

lung capacity in BPD survivors [33]. Sensitivity for future pulmonary morbidity is 0,27-0,67 and positive predictive value is 0,11-0,75 in different follow-up studies [32]. The Canadian Neonatal Network found in a recent overview that oxygen and/or respiratory support at 40 weeks postmenstrual age (PMA) best predicted ongoing respiratory and neurosensory morbidity at 18-24 months of age [34]. In many centers infants are discharged from hospital before 40 weeks PMA why an earlier time point would seem more practical. Newer modalities for evaluation and follow up of actual lung function and to assess regional ventilation and perfusion have been called for [35]. An updated version of the NIH definition has recently been published [36]. This also takes into consideration new modes of respiratory care such as high flow nasal cannula with room air and low flow nasal cannula with 100% oxygen. It also incorporates infants who die before 36 weeks PMA of pulmonary failure as these can be considered to have the worst form of BPD.

Table I. Suggested refinements to the definition of BPD [36]

Grades	Invasive IPPV*	N-CPAP, NIPPV, or nasal cannula ≥ 3 L/min	Nasal cannula flow of 1-<3 L/min	Hood O_2	Nasal cannula flow of <1 L/min
I	—	21	22-29	22-29	22-70
II	21	22-29	≥30	≥30	>70
III	>21	≥30			
III(A)	Early death (between 14 days of postnatal age and 36 weeks) owing to persistent parenchymal lung disease and respiratory failure that cannot be attributable to other neonatal morbidities (eg, necrotizing enterocolitis, intraventricular hemorrhage, redirection of care, episodes of sepsis, etc).				

*Excluding infants ventilated for primary airway disease or central respiratory control conditions. CPAP, continuous positive airway pressure; IPPV, intermittent positive pressure ventilation; N-CPAP, nasal continuous positive airway pressure; NIPPV, noninvasive positive pressure ventilation.

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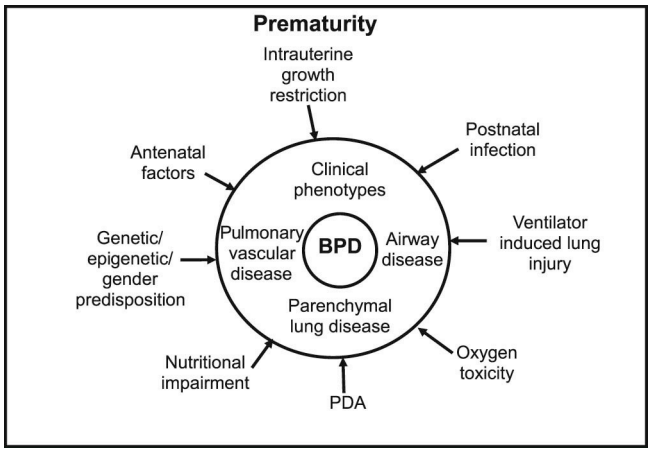
This refinement is welcome since in the NIH definition infants with CPAP are automatically classified as severe BPD. In some centers CPAP might be used for extra pulmonary conditions such as apnea of prematurity or congenital heart disease. But even in this definition no functional measurement of the lung is incorporated.

1.4 RISK FACTORS FOR BPD

The pathogenesis of BPD is multifactorial and not fully comprehended. BPD represents a wide spectrum of pulmonary disease in preterm born infants and infants with pulmonary

abnormalities. The biggest risk factor is low gestational age [37] but apart from this a wide variety of risk factors have been identified and some others have been proposed but not verified. Infants with CDH or other congenital abnormalities leading to lung hypoplasia also have a high risk of developing BPD. Figure 6 is an overview of identified and suspected risk factors.

Figure 6. Risk factors for developing BPD [36].



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1.4.1 Respiratory support

Several studies show a direct relationship between exposure to mechanical ventilation and an increased risk of developing BPD [38-42]. Compared to mechanical ventilation prophylactic nasal CPAP in very preterm infants reduces the need for mechanical ventilation, surfactant and incidence of BPD and death or BPD [43].

Early extubation to non-invasive positive pressure support after Surfactant therapy reduces days with mechanical ventilation (MV), days with supplemental oxygen and hospital stay in moderately premature infants compared to when they are exposed to prolonged MV [44]. A less aggressive ventilatory strategy at birth with initial use of CPAP instead of mandatory intubation in extremely preterm infants reduced oxygen supplementation and moderate/severe BPD or death at 40 weeks PMA in a comparison between different centers [45]. CPAP versus intubation and surfactant in the delivery room decreases pulmonary morbidity at 18-22 months [17]. Strategies aimed at avoiding mechanical ventilation in extremely preterm infants reduces rate of BPD [46]. Initiation of mechanical ventilation is an independent risk factor for developing BPD [47]. In one study mechanical ventilation on day seven had high sensitivity (99%) and specificity (67%) for predicting BPD and even higher specificity (92%) for predicting severe BPD [39]. Increasing days with MV is associated with an increasing severity grade of BPD [37].

1.4.2 Oxygen toxicity

Studies in numerous animal models have demonstrated that high levels of oxygen alone induce a BPD like disease with compromised alveolar development and pulmonary vascular remodeling [48]. Even brief exposure to high levels of oxygen during resuscitation increases the risk for BPD in premature infants [49]. However, five randomized controlled trials, including 4800 extremely preterm infants, have shown that higher saturation targets of 91-95% versus lower saturation targets of 85-89% during the hospital stay reduced mortality and necrotizing enterocolitis before discharge but there was no difference in mortality/disability, BPD, neurodevelopmental outcome, hearing loss, retinopathy of prematurity at 18-24 months [50-54]. In all five studies there was a non-significant trend for a reduction of BPD at 36 weeks in the low saturation group, which is expected since they could be weaned of oxygen earlier with lower saturation targets. According to one review the quality of evidence for these outcomes was moderate to low [55]. In a recent review by Stenson he states that saturation targets below 90% increase mortality and do not reduce morbidity such as ROP and BPD and can therefore not be recommended [56].

1.4.3 Infection

Several studies suggest a correlation between chorioamnionitis and BPD. A meta-analysis from 1996 including 59 studies and over 15,000 infants concluded that there is a limited association after adjustment for gestational age [57]. A more recent review with VLBW infants born 1989-2014 arrives at the same conclusion [58]. Chorioamnionitis induced lung maturation and increases surfactant production [59] but prenatal inflammation in the lung might make it more susceptible to postnatal injury.

Postnatal sepsis is a well-known risk factor for developing BPD [37,58,60]. However, Novotsky *et al* showed in a study of 9000 infants that treatment with antibiotics for >48 h during the first week of life increased the risk for BPD and endotracheal colonization of resistant gram-negative bacteria [61]. This raises a concern about prescribing routine prophylactic antibiotics for > 48 hours in extremely preterm infants which is common in many centers. Inflammation caused by supplemental oxygen, mechanical ventilation and infections increase pro-inflammatory cytokines in tracheal aspirates and blood samples, which have been shown to increase the risk for BPD [62].

1.4.4 Growth restriction

Several studies show that being born small for gestational age (SGA) and postnatal growth failure is a risk factor for BPD [63-69].

1.4.5 Persistent ductus arteriosus

Persistent ductus arteriosus is a risk factor for BPD and death [37,70] but treatment, medically or surgically increases the incidence of BPD [71,72]. However, late medical treatment versus early treatment reduces the risk for BPD [73]. Surgical versus medical ligation improves survival but increases morbidity and worsens neurodevelopmental outcome [74]. A recent review indicates

that if adjusting for clinical risk factors and morbidity before ligation there is no difference in outcome except for lower mortality in the group with surgical ligation [75].

1.4.6 Genetics

There is much on-going research regarding genetic susceptibility and BPD. One twin study reported that genetics contributed to approximately 80% of the observed variance in rates of BPD [76]. No genomic loci or pathways have been identified yet. The SPOCK2 gene which is a key regulator of alveolarization has been suggested while others suggest that angiogenesis related genes may be more important in the development of BPD [77,78]

1.5 TREATMENT STRATEGIES AND THEIR EFFECT ON BPD

Measures to prolong pregnancy are the most efficient way to avoid BPD. If not possible, the lung should be protected from injury and arrested development. Increased severity of respiratory failure is associated with BPD and or death [42].

1.5.1 Sustained inflation

Sustained inflation without PEEP pressure in animal models leads to a decreased effect of surfactant and lung injury [79]. In preterm infants there are contradictory findings regarding sustained inflation. There is no internationally accepted definition for sustained inflation but the term has been used in studies for inflation with peak inspiratory pressure of 20-25 cmH2O for 5-15 seconds and a PEEP of about 4-6 cm H2O. Sustained inflation compared to standard treatment (CPAP or positive pressure intermittent ventilation) is associated with shorter duration of mechanical ventilation but no reduction in morbidity including BPD or mortality or this outcome has not been studied [80-83].

1.5.2 Less invasive surfactant administration

Less invasive surfactant administration (LISA) or minimally invasive surfactant therapy (MIST) is a technique to administer surfactant to spontaneously breathing preterm infants on CPAP [84,85]. Since the lungs of premature infants are very susceptible to ventilator-induced lung injury [79,86-89], LISA has been developed to avoid mechanical ventilation in preterm infants with RDS. The most commonly described method is intratracheal surfactant deposition with a thin catheter during direct laryngoscopy.

LISA reduces need for intubation and MV [90] and compared to INSURE (Intubation surfactant administration extubation) reduces MV and BPD [91]. A meta-analysis has shown that LISA reduces BPD, BPD/death and the need for MV [92]. Comparing LISA to CPAP alone, INSURE, nasal intermittent positive pressure ventilation, nebulized surfactant administration via laryngeal mask and MV, LISA was associated with less BPD/death and severe IVH and was found to be the best strategy in extremely preterm born infants [93].

1.5.3 Mechanical ventilation

There is no consensus about optimal respiratory support for extreme preterm infants or term infants with lung hypoplasia.

In a Cochrane review with 629 infants comparing volume-targeted mechanical ventilation versus pressure-limited mechanical ventilation BPD/ death, pneumothorax, days with mechanical ventilation, hypocarbia and PVL/IVH grade 3-4 were reduced [94]. Synchronized mechanical ventilation reduces air leak and duration of mechanical ventilation but has not yet proven to decrease the incidence of BPD [95].

High frequency oscillatory ventilation (HFOV) is a ventilatory strategy that in theory can be used to avoid volutrauma caused by conventional ventilation. HFOV used with an open lung strategy will enable a more uniform lung inflation and better alveolar/ saccular recruitment. Animal studies demonstrate that HFOV gives less lung injury and improves pulmonary outcomes, compared to conventional mechanical ventilation [96,97].

Sun et al. showed that elective high frequency oscillatory ventilation compared to conventional ventilation in preterm infants reduces the rate of BPD/death, need for Surfactant, duration of mechanical ventilation, ROP>2 and moderate and severe neurological disability at 18 months [98]. In this study though, Surfactant was only given to infants with severe RDS with PaO2/FIO2 < 200 mm Hg after 2 hours of ventilation and only to infants whose parents agreed to pay.

In the last Cochrane review from 2015 comparing HFOV and conventional ventilation they concluded that the risk for BPD, severe ROP, cerebral paresis and poor mental development was decreased but risk for pneumothorax or emphysema was increased in the HFOV group [99-102]. The studies included were not consistent. All but one study showed a significant reduction in oxygen need at 28 or 30 days. Some used a strict lung volume recruitment strategy targeting at very low level of supplemental oxygen while others had a higher or unspecified FiO2 target [103]. Adolescent survivors who had been treated with HFOV in infancy had superior small airway function measured with pulmonary function testing and higher ratings from teachers when compared to infants treated with conventional ventilation [104].

It has been shown that an earlier first extubation attempt leads to a shorter length of stay and less risk for BPD regardless if the infant required re-intubation [105]. Total duration of mechanical ventilation was more predictive of BPD than courses of mechanical ventilation [106].

1.5.4 Hypocapnia

Hypocapnia as an indicator of more aggressive respiratory management has been suggested as a risk factor for BPD. In one study patients with pCO2<5.3 kPa at 48 h age were more likely to develop BPD than infants with pCO2>6.6 kPa and in another study infants with hypocapnia before Surfactant treatment were more likely to develop BPD [107,108]. Hypocapnia

will lead to a respiratory alkalosis and induces cerebral vasoconstriction with decreased oxygen delivery within the brain and increases neuronal excitability. It increases the risk for periventricular leukomalacia possibly because of hypo perfusion. When the partial pressure of pCO2 normalizes the vasodilatation and increased perfusion might increase the risk of intraventricular hemorrhage [109].

In the lung hypocapnia induces bronchospasm, increases pulmonary-capillary permeability and decreases lung compliance and might affect surfactant function, which results in increased airway resistance and increased work of breathing. Alveolar hypocapnia also attenuates vasoconstriction caused by hypoxia [110].

1.5.5 Hypercapnia

Hypercapnia has been suggested to reduce ventilator-induced injury and BPD [111]. Permissive hypercapnia during the first weeks of life to avoid intubation and enable earlier extubation seems to reduce BPD [46]. However, permissive hypercapnia in mechanically ventilated extreme preterm infants has not been proven to reduce the rate of moderate/ severe BPD or death [112,113]. Some studies even suggests that hypercapnia during mechanical ventilation increases the rate of BPD [114]. In another study hypercapnia was also correlated to higher incidence of mortality, moderate/ severe BPD, NEC and a poorer neurodevelopmental outcome but after adjusting for respiratory index: mean airway pressure x fraction inspired oxygen (MAPxFiO2) and birth weight it was no longer significant. The authors conclude that hypercapnia might be a marker for disease severity [115]. In some studies permissive hypercapnia seems to be lung protective and safe but the clinical benefits are modest [111]. Other studies indicate that higher and fluctuating values of pCO2 are an independent predictor of IVH/death, BPD/death and NDI/death in extreme preterm infants [116]. The last Cochrane review performed in 2001 did not find enough evidence to recommend permissive hypercapnia [113]. Nevertheless, marginal increase in pCO2 level from 6.4 kPa (48 mmHg) to 6.93 kPa (52 mmHg) leads to a significant reduction in days on mechanical ventilation [117,118].

In an animal study though, hypercapnic acidosis increased pulmonary pressure even after normalizing the pH value with buffer [119]. In vitro models show that alveolar fluid clearance and repair of epithelial cell membranes in the lung are impaired by hypercapnia [120,121]. Hypercapnia reduced functional vessel density in the skin at two weeks of age with fewer small vessels but more large vessels [122]. Direct effects of hypercapnia within the lung in infants have not been studied.

1.5.6 Fluid and nutrition

Higher volumes of fluid and less weight loss during the first 10 days of life are risk factors for developing BPD [37] but so is poor postnatal growth. A balance must be achieved to provide enough calories without fluid overload. 180 ml/kg/day of standard formula compared to 145 ml/kg/day concentrated formula from 28 days of age in infants with BPD did not reduce

oxygen requirement at 36 weeks postmenstrual age [123]. Fluid restriction is controversial and adequate nutritional intake must be taken into consideration.

Furosemide improve short term respiratory function and might be considered to enable extubation or avoid reintubation but has no effect on long term pulmonary function or incidence or severity grade of BPD [124,125].

Early nutrition is essential. Malnutrition increases incidence and severity grade of BPD [69]. A minimum of 2 grams protein/kilo and 2 gram of lipids/kilo should be given the first day with an increase to 3.4-4.0 grams/kg/day within 72 hours [126]. Energy needs for infants with BPD are 15-25% higher than in infants without BPD. A daily energy intake of 140-150 kcal/kg/day is recommended [127]. Early excess weight gain must be avoided thought since this increase the risk for later insulin resistance and metabolic disease [128].

1.5.7 Pharmacological interventions

1.5.7.1 Vitamin A

Intramuscular injection of vitamin A three times a week reduces BPD/ death with 7% [129]. In animal models vitamin A or retinoid acid attenuates lung injury and alveolar simplification and promotes alveolarization [130]. It has not been widespread clinically because of modest effect and the risk and discomfort of multiple intramuscular injections.

1.5.7.2 Steroids

Steroids have been used to reduce inflammation and induce lung maturation but have side effects. Steroids alter weight gain velocity with a decrease during and after treatment and a later catch-up during the first two years of life. Head circumference growth is delayed but normalized at one year of age in boys but not normalized in girls [131]. In animal models it reduces alveolar surface area [130]. Follow-up studies have shown increased risk of neurodevelopmental impairment at two years of age [132], especially cerebral palsy [133], and decreased brain volume in adolescence [134]. After reports like these the use of postnatal systemic steroids in very low birth weight infants decreased from around 20% per year 1997-2000 to about 8% presently in the US.

More recent reviews showed a decreased risk for BPD and improved neurodevelopmental outcome for infants receiving high cumulative doses of Dexamethasone (> 4 mg/kg) compared to infants receiving moderate doses (2-4 mg/kg) or low doses (<2 mg/kg). Early administration (started <8 days of age) compared to moderately early (started at 8-21 days of age) or delayed (started >21 days of age) did not alter outcome of BDP, death or BPD or later neurodevelopmental impairment [135] In a Cochrane review from 2017 they conclude that compared to placebo, early systemic steroids (before 8 days of age) decreased rate of BPD/death or BPD at 28 days and 36 weeks postmenstrual age. It improved neurodevelopmental outcome at 2 years of age in some studies but not in others. Steroids reduced the incidence of patent ducts arteriosus and ROP but had short-term adverse effects

with increased risk for gastrointestinal perforation, hyperglycemia, hypertension, hypertrophic cardiomyopathy and growth failure [136].

Late steroid treatment (started after 7 days of age) reduced BPD at 28 days and 36 weeks postmenstrual age without increasing neurological impairment at late follow-up [137]. It reduces mortality at 28 days but not at 36 weeks postmenstrual age. It decreases death/BPD at both 28 days and 36 weeks. There is a trend for increase in infections and gastrointestinal bleeding but not in NEC and a trend for reduction in severe IVH. There was also an increase in severe ROP but not blindness. There was no substantial difference in neurological outcome or respiratory health and function at two years of age but later in childhood a significant decrease in FEV1 in the Dexamethasone group [137].

In the PREMILOC study 523 infants were given a low dose of Hydrocortisone for the first 10 days of life, total dose 8.5 mg/kg. This increased the rate of survival without BPD at 36 weeks postmenstrual age and had no adverse effect on neurodevelopmental outcome at 22 months of corrected age [138,139]. Another study with early but 30% higher doses of hydrocortisone showed that at 5-7 years of age 61% of the children in the hydrocortisone group had some neurodevelopmental impairment but only 39% in the placebo group. This study included 18 cases and 19 controls at follow-up [140].

Inhaled corticosteroids starting before seven days of age have been shown to decrease BPD or death at 36 weeks postmenstrual age [141]. There was no difference in neurodevelopmental outcome at two years of age but an increase in mortality in the budesonide group [142]. Inhaled corticosteroids started after seven days of age increased probability of successful extubation but there was no difference in rate of BPD, mortality or days with mechanical ventilation [143]. There is one promising study showing that installation of budesonide together with surfactant reduces additional doses of surfactant needed and incidence of BPD or death/BPD. Levels of interleukins in tracheal aspirate were also lower than in infants only receiving surfactant. No adverse effects including differences in neurodevelopmental outcome at 2 years of age were noted. In this study though, up to six doses of surfactant and budesonide were given [144].

1.5.7.3 Bronchodilators

Salbutamol increases short-term lung mechanics but there is no reduction in BPD or mortality [145,146]. One small study showed a significant reduction of oxygen dependency and BPD in infants treated with aminophylline the first 10 days of life [147]. The quality of evidence in this study was graded as very low.

1.5.7.4 Nitric oxide

Inhalation of nitric oxide has no proven effect in premature infants with or without pulmonary hypoplasia as rescue treatment, prevention or treatment for incidence of BPD or BPD/death [148-150].

1.5.7.5 Caffeine

Caffeine was initially used for prevention of apnea of prematurity but several studies have shown that it also increases the rate of successful extubations, decreases days with MV, reduces the rate of BPD and improves neurodevelopmental outcome at 1, 2 and 5 years of age [151-155]. There is some evidence that a higher maintenance dose reduces the incidence of BPD or death/BPD and improves neurodevelopmental outcome but these results must be verified in a larger study before a general recommendation can be given [156].

1.6 COMPLICATIONS OF BPD

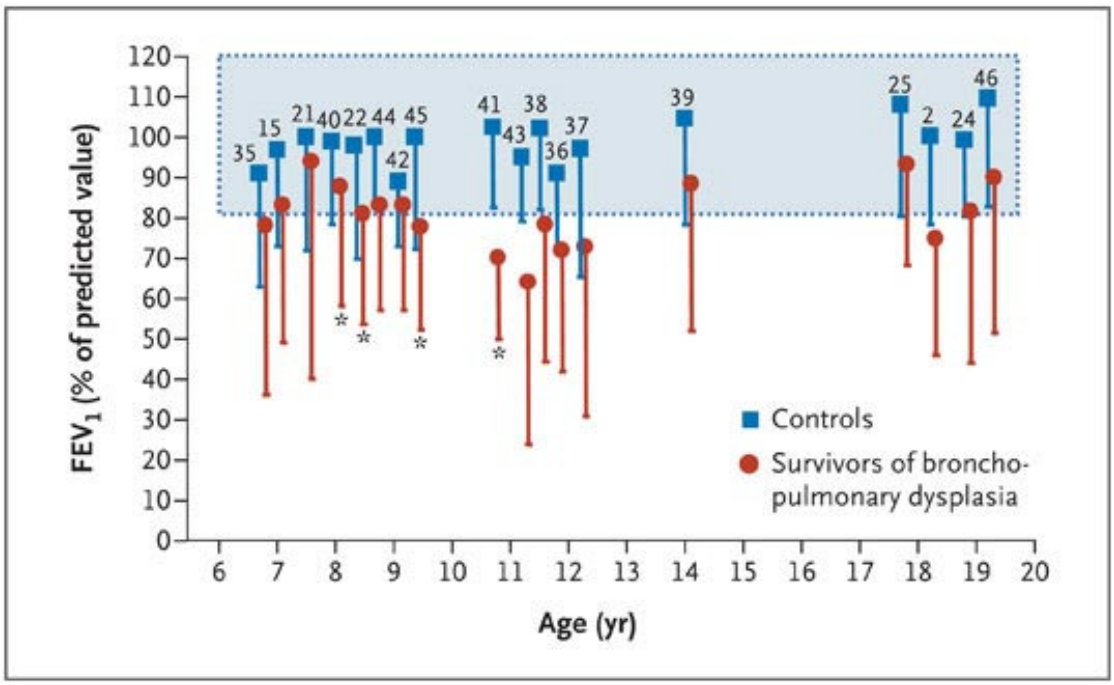
BPD increases the risk for growth failure, pulmonary hypertension, neurodevelopmental delay, hearing defects and retinopathy of prematurity [157]. Rehospitalization because of respiratory symptoms is common the first two years of life [158]. Children with BPD have lower IQ, more academic difficulties and delayed speech and language development compared to preterm children without BPD [159].

1.7 LONG-TERM LUNG FUNCTION

Children with BPD often report persistent respiratory problems at school age and in adult life [6,160]. Lung function in these children and adults is usually evaluated with pulmonary function testing (PFT) or reported symptoms only. PFT mainly measures small airway function while New BPD affects the distal unit with reduced alveolar surface and altered vascularization. However, children with BPD have a sustained impairment in lung function, measured with conventional pulmonary lung function testing (PFT), during childhood and adolescence [161-165].

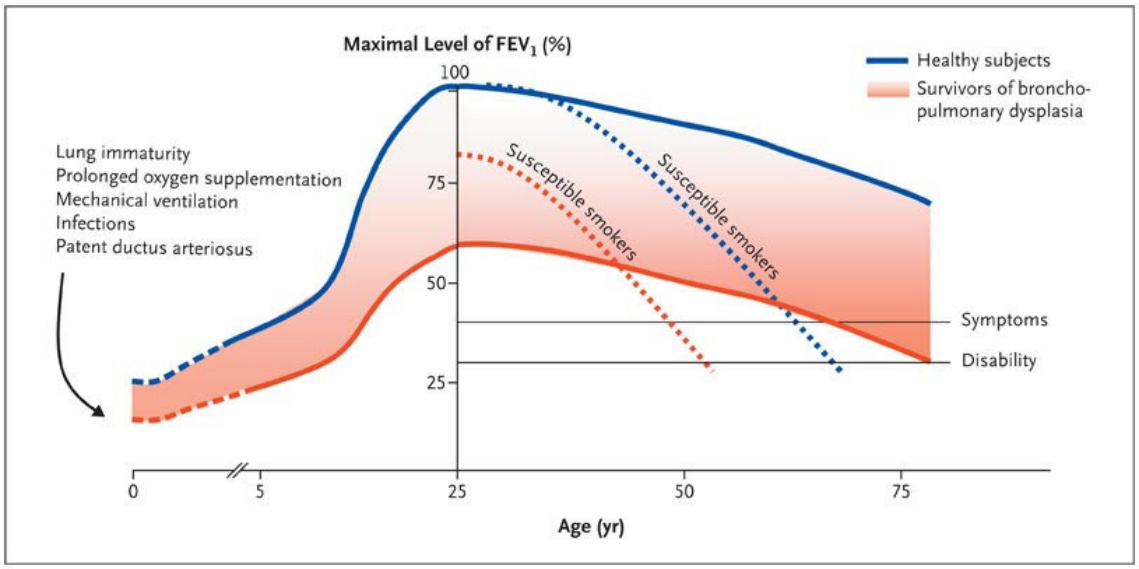
Longitudinal studies in one cohort of children show little improvement in pulmonary function testing between 2 and 8 years of age suggesting an irreversible early airway-remodeling process [166]. Other studies though indicate that alveolarization can continue up to adult age [5]. Studies in adult survivors of preterm birth demonstrate that they have a capillary rarefaction [167]. How this is related to vascularization and ventilation perfusion matching within the lung is unknown. Figure 7 is a summary of studies illustrating the difference between FEV1 values in survivors with BPD and normal controls at different ages.

Figure 7. [6] FEV1% in preterm born survivors of BPD in childhood, adolescent and young adulthood compared with full term born individuals at the same age.



In healthy subject lung function increases until the age of around 25 years of age. Then it progressively decreases. In preterm born survivors the maximum capacity measured with spirometry will be less and in preterm born survivors with BPD the plateau will be even lower [162]. In smokers the deterioration will be faster than in healthy persons. Little is known about the deterioration rate in preterm born survivors with and without BPD. Data from a large cohort of patients with very low birth weight indicates that lung function in subjects with BPD may be deteriorating at a more rapid rate than in low birth weight subjects without BPD and compared to reference values [163]. Figure 8 shows a theoretical model of development of FEV1 in individuals with BPD and healthy subjects according to age. The dashed lines represent the potential effect of smoking.

Figure 8 [6] Theoretical development of FEV1 in individuals with BPD compared to healthy individuals according to age



1.8 IMAGING TECHNIQUES AND EVALUATION OF LUNG FUNCTION IN INFANTS AND CHILDREN WITH BPD

It is difficult to evaluate lung function in infants. Chest X-ray, CT scan and conventional magnetic resonance imaging (MRI) examination provides an anatomical image but gives no information about actual lung function. Functional MRI has been used in experimental settings in children but not infants. Pulmonary function tests in infants are investigator dependent and therefore not appropriate for standardized grading of BPD.

1.8.1 Chest X-ray

Chest X-ray is easily accessible and is used for clinical evaluation on a day-to-day basis for acute complications of BPD such as edema, atelectasis, hyperinflation and lobar emphysema. However it gives only marginal information about severity of BPD since many patients with new BPD have almost normal chest X-rays despite significant respiratory symptom [168].

1.8.2 Computerized tomography

Several studies using High Resolution Computerized Tomography have demonstrated structural pulmonary abnormalities in preschool children, older children and young adults with BPD [169-173]. The clinical implication of these abnormalities is unknown. The high radiation dose is also a concern. It has not been used in infants for systematic evaluation. These studies support the notion that BPD is a disease of the peripheral airways with arrested development and alveolar simplification and has limited central airway involvement [170,174]. Some studies using CT show a correlation with BPD grade, days with mechanical ventilation

and pulmonary function testing but serial studies with CT is not an option because of increased risk for cancer [175].

1.8.3 Conventional Magnetic resonance imaging.

MRI has the advantage of no radiation. The major drawback of conventional MRI is that imaging of the neonatal lung is difficult since proton density is low and motion artifacts from rapid cardiac and respiratory motion degrade quality of the image. The equipment is large and expensive and each examination takes a long time. MRI in infants is feasible, preferably with a smaller specially designed MRI camera [176,177]. Structural abnormalities on MRI at term age graded according to an MRI severity score was correlated with clinical BPD grading and predictive of respiratory support at discharge[178]. Infants with BPD have higher proton density than term controls but the clinical implication of this is unclear since muscle mass and fat composition might differ between preterm and term infants and differentiation of causes to high signaling is impossible. Fibrosis and atelectasis can give the same signal but have different implications for future pulmonary function. Long-term follow-up has not been reported yet.

1.8.4 Functional MRI

For adults and children with cystic fibrosis several experimental MRI techniques have been described including contrast with gadolinium chelate, magnetic "tagging" of inflowing blood, ultra-short echo time using 100% oxygen to map ventilation and blood flow to map perfusion, hyperpolarized MRI using ^{3}He or ^{129}Xe to image ventilation, diffusion and in some cases the dissolving phase in the alveoli-capillary membrane. Patient cooperation and breath holding maneuvers are required in most cases [179-183]. These are promising techniques but further work need to be done regarding reproducibility and clinical significance before MRI can become a part of routine clinical care. Hyperpolarized gas MRI is not clinically available due to limited access, high costs and regulations regarding noble gases. Polarizers for noble gases are only available at a few specialized centers. In a review Fain states that the hyperpolarized MRI techniques, reported for the first time about 30 years ago, need to become more quantitative, sensitive and accessible to justify their current cost and complexity [184].

1.8.5 Non-invasive measurement of reduced ventilation: perfusion ratio

A non-invasive method of V/Q and shunt estimation has been developed using arterial oxygen saturation (SpO2) and partial pressure of inspired oxygen (PIO2) measurements with the normal hemoglobin dissociation curve as the reference point. Using this method Quine et al have stated that V/Q mismatch is the predominant cause of gas exchange impairment in infants with BPD [185]. This method has not been validated to V/Q scintigraphy yet.

1.8.6 Spirometry

Spirometry mainly evaluates small airway function while BPD is a disease of the distal respiratory units, with abnormal alveolar and vascular development [26]. Spirometry is also difficult to perform and interpret in unstable infants and investigator dependent.

1.8.7 Multi inert gas elimination technique

MIGET is the golden standard for measuring ventilation perfusion matching [186]. It is a complicated procedure and not clinically available. Six different inert gases are infused and later measured in blood and expired breath. A pulmonary artery catheter, arterial line and a venous line are required and measurement of expired gases. In healthy individuals, children and adults, the normal lung physiology studied with MIGET shows a log-normal V/Q ratio distribution centered around 1 with SD 0.4 and minimal V/Q mismatch [186,187].

1.8.8 Ventilation and perfusion Scintegraphy

The ratio of ventilation to blood flow is an important determinant for regional gas exchange in the lung. The first studies using radioactive gas, ^{133}Xenon, were performed by Knipping in Germany in 1955 to evaluate lung function in patients with lung cancer [188]. Taplin *et al* developed macro aggregated albumin particles labeled with I^{131} to measure regional pulmonary blood flow and Wagner applied perfusion scanning to the diagnosis of pulmonary embolism [189,190]. In adult medicine ventilation/perfusion scanning is mainly used for the diagnosis of pulmonary embolism but other areas for diagnostic use are developing.

V/Q relationships in BPD are not widely studied in the literature. Moylan and Shannon used ^{133}Xenon intravenously in 1979 to map perfusion and ventilation in four regions of the lung in eight BPD patients with lobar hyperinflation who could not be weaned from mechanical ventilation. Ventilation was measured by clearance of intravenously administered ^{133}Xenon why it was actually the combined capillary alveolar diffusion and the ventilation that was measured. ^{133}Xenon has a half-life of 1 minute, which limits time for registration and hence gives a low resolution. They concluded that ventilation was reduced in the affected lobe but not perfusion with severe V/Q mismatch as a result. They suggested that there was a lack of reflexive vasoconstriction in the poorly ventilated areas. One explanation might be that the infants received 40% oxygen during the examination. High levels of oxygen might aggravate V/Q mismatch [191]. Murray et al report V/Q scans in three infants with BPD and lobar hyperinflation. They came to another conclusion. Ventilation was mapped with ^{81m}Kr. All had normal ventilation in the affected lobe, some perfusion but severe V/Q mismatch in other collapsed regions of the lung [192]. ^{81m}Kr is a gas with a half-life of only 13 seconds, which gives a low resolution and requires patient cooperation for image optimization, which might have influenced the results. Soler et al showed a good correlation between clinical severity score, roentgenographic scoring [23] and two-dimensional perfusion scintigraphy at 6 months of age in infants with BPD compared to controls [193].

Planar perfusion and ventilation scans have also been used in children with congenital malformation to evaluate lung function before lobectomy and in follow up studies in patients with congenital diaphragmatic hernia [194-197]. In individuals with CDH perfusion and ventilation in the ipsilateral lung compared to the contralateral lung are reduced during infancy [194,195]. Ventilation gradually normalizes in the majority of the patients during childhood but there is a sustained reduction in perfusion [194-197] and a resulting V/Q mismatch [198]. It has been suggested that all CDH patients should be followed with planar V/Q scans since in some patients severe and progressive V/Q mismatch, especially on the vascular side, develops with increasing age [199]. V/Q mismatch itself has also been demonstrated to be a sensitive predictor of future pulmonary morbidities such as obstructive pulmonary disease [196,198]. In cystic fibrosis skewness in V/Q matching between different parts of the lung correlated with FEV1 and disease severity [200].

1.8.9 Single photon emission computed tomography

Single photon emission computed tomography (SPECT) is a three dimensional scintegraphic technique that can estimates actual lung function in different parts of the lung based on regional ventilation (V) perfusion (P) matching. V/Q SPECT has the unique advantage of imaging functional changes at regional level in detail. It quantifies proportion of functional loss caused by matched defects (reduced ventilation and perfusion), mismatched areas (reduced perfusion compared to ventilation) or reversed mismatched areas (reduced ventilation compared to perfusion) [201]. SPECT has also proven to be a sensitive method for detecting perfusion abnormalities within the lung in other pulmonary diseases in adults [202].

Compared to the planar V/Q scint segmental overlap and shine-through of the adjacent lung is avoided and the location and size of perfusion and or ventilation defects in individual segments can be more accurately defined [202]. SPECT has higher sensitivity, specificity and accuracy than planar imaging [203].

SPECT has not been done previously in infants because of a high radiation dose, the lack of appropriate agent for inhalation to map ventilation and a lack of techniques for administration of an inhaled agent. Our new technology allows quantification of the regional distribution of ventilation (V) and perfusion (Q) and matching between them (V/Q match) in the lungs of infants with high resolution and a low radiation dose [204]. To increase time for acquisition a Technetium-labeled aerosols with a stable deposition within the alveolar sac can be used to image ventilation.

Reference values for SPECT V/Q matching for healthy individuals are difficult to find in the literature. Some studies have defined lung volume with V/Q ratio matching [0.8-1.2] as matched regions and areas outside of this range as mismatched or reverse mismatched. Others have defined matched regions as regions with V/Q ratio [0.6-1.4]. Combining data from our institution with other studies we conclude that for healthy individuals with anatomically and functionally normal lungs, the lung volume with good V/Q ratio matching [0.6-1.4] should cover more than 89% of the total lung [201,205-208]. According to the current knowledge on

normal pulmonary physiology evaluated by multiple inert gas elimination technique (MIGET), the golden standard for evaluation V/Q matching, lung volume with normal V/Q matching should be >89% [186]. V/Q ratios measured with SPECT technique are in good agreement with MIGET (r2=0,99) [209].

2 AIM

The overall aim of this thesis was to complement the clinical grading of BPD with a functional imaging to better predict lung function morbidity during childhood and investigate the correlation between early respiratory strategies and later lung function impairment.

The specific aims were:

Paper I and II

-To quantify the extent of lung function impairment estimated by ventilation/perfusion (V/Q) matching and correlate this with the current clinical severity grading for BPD and clinical disease severity markers for CDH.

Paper I and II

-To investigate whether V/Q abnormalities correlates with duration of respiratory support and need for supplemental oxygen

Paper III

-To study whether children who were diagnosed with BPD in infancy have persistent V/Q abnormalities at 10 years of age measured by SPECT and to correlate the abnormalities with clinical grading and pulmonary function testing

Paper IV

-To investigate if disease severity score and respiratory management during the first week of life correlates with V/Q abnormalities and clinical BPD grading at full-term

3 MATERIALS AND METHODS

3.1 SUBJECTS AND STUDY DESIGN

This was a cross-sectional study of preterm born subjects with BPD and subjects with congenital diaphragmatic hernia and suspected lung hypoplasia. All patients in study I, II and IV were admitted to Karolinska University Hospital between 2006-2010. Patients in study III were admitted to the Karolinska Hospital or Södersjukhuset in Stockholm between 1998-1999.

3.1.1 Paper I and IV

The criteria for patient inclusion was a clinical diagnosis of BPD, oxygen requirement at 28 days of age [29]. Also, the infant had to be sufficiently stable to tolerate a transport to the Nuclear Medicine facility (5 minutes away) and to have had a recent negative echocardiogram excluding a right-to-left heart shunt. This was to minimize the risk of leakage of macro aggregate human albumin (MAA) particles to other organs. A total of 32 infants were eligible after written and verbal parental consent. Clinical grading was performed at 36 weeks postmenstrual age. Three-dimensional Single photon emission computed tomography (SPECT) was scheduled as soon as possible after the BPD clinical grading was performed. Median age at SPECT was 37 weeks postmenstrual age. SPECT analysis data from 2 patients was excluded due to body movement artifacts. At 36 weeks all infants in the mild group (n=9) breathed room air with a saturation of >90%. All patients who required oxygen 22-30% and were free from positive pressure support failed the oxygen reduction test and were graded as Moderate BPD (n=11) [30,31]. All patients in the severe group were on CPAP at 36 weeks PMA (n=10) and 5 were still on CPAP at the time of the SPECT investigation. There was no significant difference in PMA at examination between the severity groups.

3.1.2 Paper II

Twelve infants with CDH were consecutively recruited during 2006-2008. Mean GA at birth was 37 weeks (range 35-41 weeks). All patients underwent surgical repair of CDH in the newborn period. At the time of the SPECT examination the patients were on average 6 month of age (range 3-12 months). The hernia was left sided in 10 patients and right sided in 2 patients. 9 of the patients had patch repair and 8 had large defects. None of the patients had congenital heart defects or chromosomal abnormalities. 6 patients required ECMO and 8 infants had pulmonary artery hypertension in the postoperative period. Two patients required supplemental oxygen at the time of SPECT. 6 patients had no BPD, 4 had mild BPD, and 2 had severe BPD.

3.1.3 Paper III

A hospital-based prospective register of neonates with breathing difficulties in Stockholm County, was used to identify children with a diagnosis of BPD. Children who were born between January 1998 and December 1999 were invited to participate (n=75) in the PrematUre follow up with Lung function Mannitol and Metacholine study (PULLM) at the Karolinska University Hospital. 35 children with BPD accepted to participate in the PULLM study out of which 30 completed all studies in PULLM and 26 of these agreed to also participate in the SPECT study. Of the 4 patients who declined participation in the SPECT study, 3 had mild BPD and 1 moderate or severe BPD. Out of the 26 children who participated, 10 were graded as mild BPD in infancy, 10 as moderate and 6 as severe BPD respectively. The mean age at examination was 10.1 years (SD 0.96 years). All children attended regular schools and had no asthma symptoms at the time of the SPECT examination. There were no significant differences in GA, sex, grade of BPD, days with mechanical ventilation, days with CPAP, days with supplemental oxygen and APGAR score at five minutes between the excluded and included patients.

3.2 METHODS

Method for imaging infants with 3-D SPECT made possible by:

Higher resolution

Lower dose isotope needed

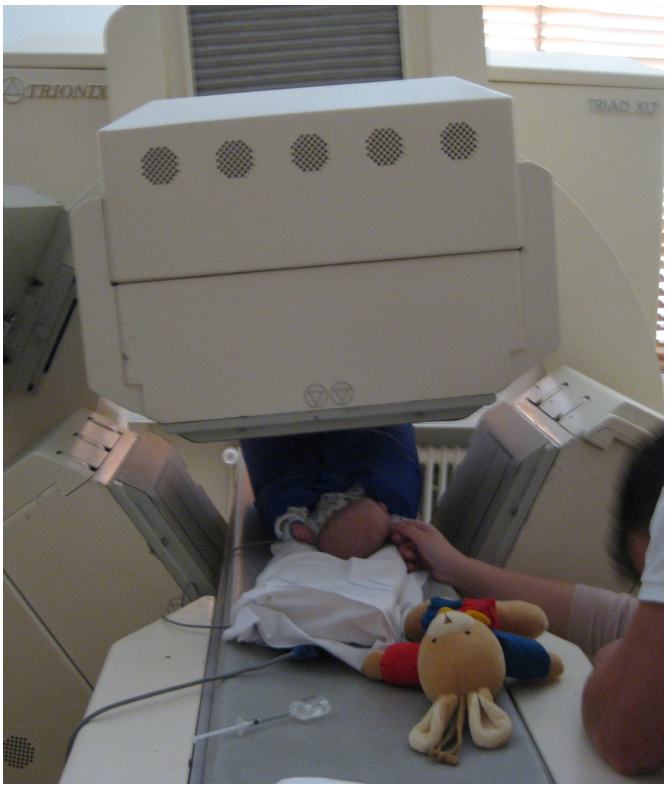
Ventilation:

^{99m}Tc-Technegas- ultra-fine aerosol with stable particle deposition within alveolar sacs

Passive inhalation with nCPAP –no patient cooperation needed

Perfusion mapped with 99mTc marked Albumin

Examination performed in quiet sleep. Patient immobilized in a vacuum mattress



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3.2.1 Single photon emission computed tomography

3.2.1.1 Examination

The SPECT examinations were performed at the Department of nuclear medicine, Karolinska University Hospital following the protocol previously described by our group [204]. A three-headed gamma camera (TRIAD XLT, Trionix Research laboratory, Twinsburg, OH, United States) equipped with low-energy, high-resolution parallel-hole collimators was used. SPECT acquisitions were performed with 90 projections, 30 sec per projection (15 min total acquisition time) and 128x128 image matrix size with a pixel size of 3.56 mm^2. Perfusion SPECT scan was performed after intravenous administration of approximately 3-8 Mega-Becquerel (MBq) of Technetium 99m labeled macro aggregate albumin (Tc99m-MAA; Mallinckrodt Medical, Petten, The Netherlands) according to age and weight. Thereafter, with the subject lying in the same position, the ventilation SPECT scan was started after administration of approximately 5-15 MBq of Technegas aerosol (Tetley Manufacturing Ltd. Sydney, Australia) according to age and weight during normal tidal breathing. Oxygen saturation and heart rate were continuously monitored with a pulse oximeter during the entire procedure. The contribution from Tc99m-MAA in the ventilation scan was removed by pixel wise subtraction in the projection plane. An ordered subset expectation maximization (OSEM) reconstruction algorithm with 8 subsets was used for image reconstruction of the perfusion, ventilation and combined SPECT projection data. Subsequently three sets of reconstructed transversal slices were produced. The patient received an estimated total dose of 0.7-1.5 mille-Sievert.

3.2.1.2 SPECT image quantification and data analysis

SPECT image quantification was performed using specially developed software for data analysis (Matlab and Image Processing Toolbox Release R2015a, The MathWorks, Inc., Natick, Massachusetts, United States). For each patient, the lung volume was first segmented using the combined ventilation perfusion reconstructed images. This ensured that all functional lung tissue was considered. Image lung segmentation was then performed with a region-growing algorithm with a seed located at the center of the lungs. Thereafter, the V and Q images were normalized to their average and the pixel-wise ventilation perfusion ratios (V/Q ratios) calculated. V /Q mismatch was used to quantify the extent of lung function impairment.

V/Q ratio interval [0.6-1.4] was defined as a matched V/Q ratio. The presence of regionally reduced ventilation and/or perfusion matching was assessed outside of this V/Q ratio interval and described as mismatched if V/Q >1.4 (Q <<V) or reverse mismatched if V/Q < 0.6 (V<<Q). The extent of these three V/Q functional regions was expressed as a percentage of the total lung volume and scored for each patient at a BPD group level. In preterm infants we divided the group in two according to SPECT findings. V/Q matching in less than 50% of the lung volume was defined as unsatisfactory SPECT and V/Q matching in more than 50% of the lung was defined as satisfactory SPECT. This value was set very low based on findings

that all very premature infants, even ones without BPD, have a reduced lung function [19]. In infants with CDH we compared the ipsilateral lung (the same side as the hernia), with the contralateral lung (opposite side) as this had been done earlier in studies with planar ventilation perfusion scintegraphy. Infants where the lung volume of the ipsilateral lung (Vol_i) was less than 50% of the contralateral lung (Vol_c) were compare with infants with Vol_i /Vol_c>0.5. In 10-year-old children with BPD the V/Q ratios were also re-sampled into 50 equally spaced log V/Q ratio compartments to be able to compare to MIGET. Fractional distribution of V and Q 0.1-1, 1-10 and >10 was also scored.

3.2.2 Clinical records

Clinical parameters for all patients including gestational age, birth weight, premature rupture of membranes, APGAR score, SGA, days on MV, CPAP and supplemental oxygen were collected. Also details for postnatal age at intubation and surfactant administration, uneven distribution of surfactant and daily, for the first seven days of life, values for pCO2 maximum, minimum and fluctuations, MAP, FiO2, days with MV the first week of life, oxygenation index and CRIB score (clinical risk index for babies) were collected for premature infants with BPD. Days with ECMO and persistence of pulmonary hypertension were recorded for infants with CDH. Pulmonary function test findings, medication for asthma and result on Asthma Control Test were recorded for children born preterm with BPD.

3.3 STATISTICAL ANALYSIS

In paper I the statistical analysis was performed using MATLAB. Student's t-test was used for continuous measures. Regression was used to identify predictors of V/Q abnormalities. A correlation for gestational age was applied.

In paper II the SPECT data was compared between the right and left lung using non-parametric Wilcoxon matched-pairs signed-ranks test. Student's t-test was used for continuous measures when comparing groups. Regression was used to identify predictors regarding the variance in outcome.

In paper III the Kruskal Wallis test, Mann-Whitney U test and Student's t-test were used for continuous measures where appropriate.

In paper IV the Kruskal Wallis test and Mann-Whitney U tests were used where appropriate to compare BPD groups, clinical parameters and V/Q matching. The material was normally distributed except for oxygenation index so parametric methods could have been used in all statistical calculation like we did in paper I but due to the small sample size and many parameters analyzed we decided to also use non-parametric tests. Linear Mixed Model was used for continuous repeated measures and clinical and demographic explanatory variables. An interaction term was introduced in the model to examine heterogeneity effect. We also used Generalized Estimating Equation to analyze ordinal outcome variables, to control for

potential confounders and to examine the fixed effect of gender. Multiple linear regression analysis was employed to examine the relationship and control for potential confounders for independent samples. For all statistical analysis, $P<0.05$ was considered significant.

3.4 ETHICAL CONSIDERATIONS

The regional ethical committee in Stockholm, and the radiation protection committee at Karolinska University Hospital approved all studies included in this thesis and written and verbal consent was obtained from all parents of the participating infants and children. Individual results of the SPECT examination were given to the parents by the investigator or the clinician responsible for the patient. The examination involved no discomfort for the subjects and no severe adverse events were reported

.

4 RESULTS AND DISCUSSION

In summary all the studies reveal that infants and children with BPD or CDH have widespread V/Q abnormalities (Figure 1). Lung function measured with SPECT is negatively correlated with days on mechanical ventilation in infants but not in 10-year-old children. A need for supplemental oxygen at 36 weeks postmenstrual age in preterm infants and at 28 days and 56 days of age in infants with CDH is correlated with V/Q abnormalities. In children there was a non-significant trend for reduced V/Q matching with increasing severity grade of BPD.

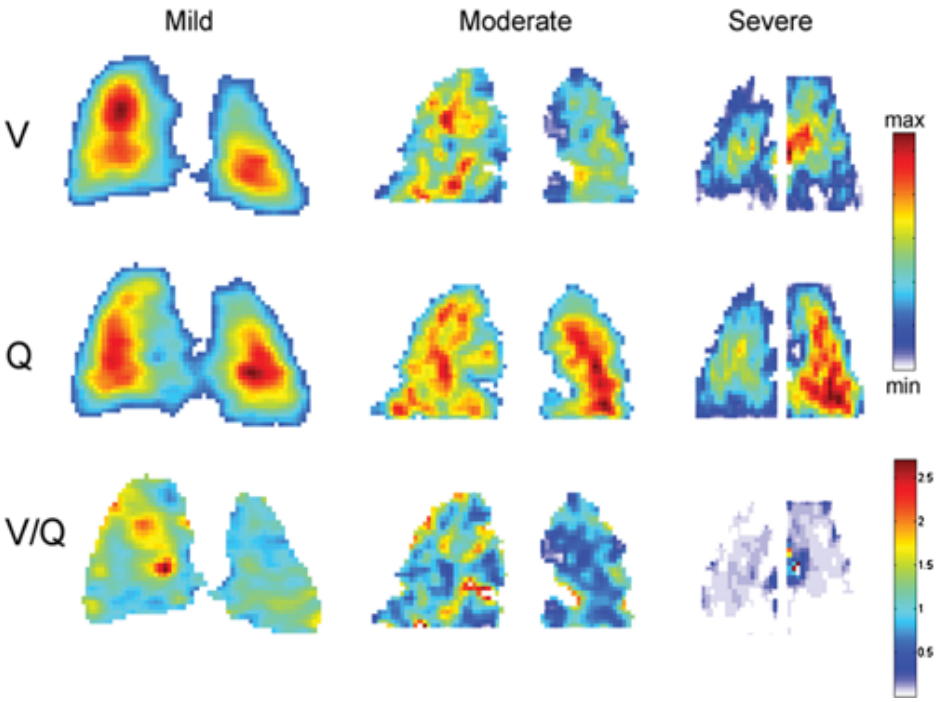


Figure 1. Representative coronal SPECT slices of lung ventilation (V) and perfusion (Q), and corresponding ratio (V/Q) for one individual from each BPD group of preterm born infants. Left panel, mild BPD patient showing a good V/Q matching in both lungs. Middle panel, moderate BPD with more uneven V/Q matching. Right panel, severe BPD with widespread abnormalities in both ventilation and perfusion and low matching between the two.

4.1 LUNG FUNCTION

In study I, preterm infants with BPD, only 54% (range 28%-76%) of the total lung volume had satisfactory V/Q matching. In study II, in infants with CDH, the V/Q matching in the lung on the hernia side was 38% (range not measurable-74%) and on the contralateral side 56% (range 36%-73%). In some patients the hernia side lung received as little as 3% of total ventilation and perfusion. In the CDH group as a whole the ipsilateral lung contributed to 30% of the ventilation and 28% of the perfusion. In Figure 2 three examples of distribution between the lungs are show.

In preterm born children with BPD, now at school age, approximately 62% (range 46%-82%) of the total lung volume had good V/Q matching.

In infants with BPD there was a left shift of the V/Q distribution curve with an increasing severity grade of BPD with ventilation being more affected than perfusion. In the 10-year-old preterm born children with BPD it was the opposite with a right shift of the curve for the whole group with reduced perfusion contributing more to the V/Q abnormalities. Lung volume with mismatch (V>Q) was larger compared to areas with revere mismatch (Q>V), 26.2% and 11.8% respectively (Figure 3.) This was also true for some of the infants with CDH with very low perfusion in the ipsilateral lung.

Expressing lung function as V to V/Q and Q to V/Q we found that in 10-year-old children with BPD the fractional distribution of ventilation and perfusion to V/Q was reduced compared to lung healthy children at the same age, V to V/Q was 31% compared to 64% respectively and Q to V/Q 51% compared to 89% respectively.

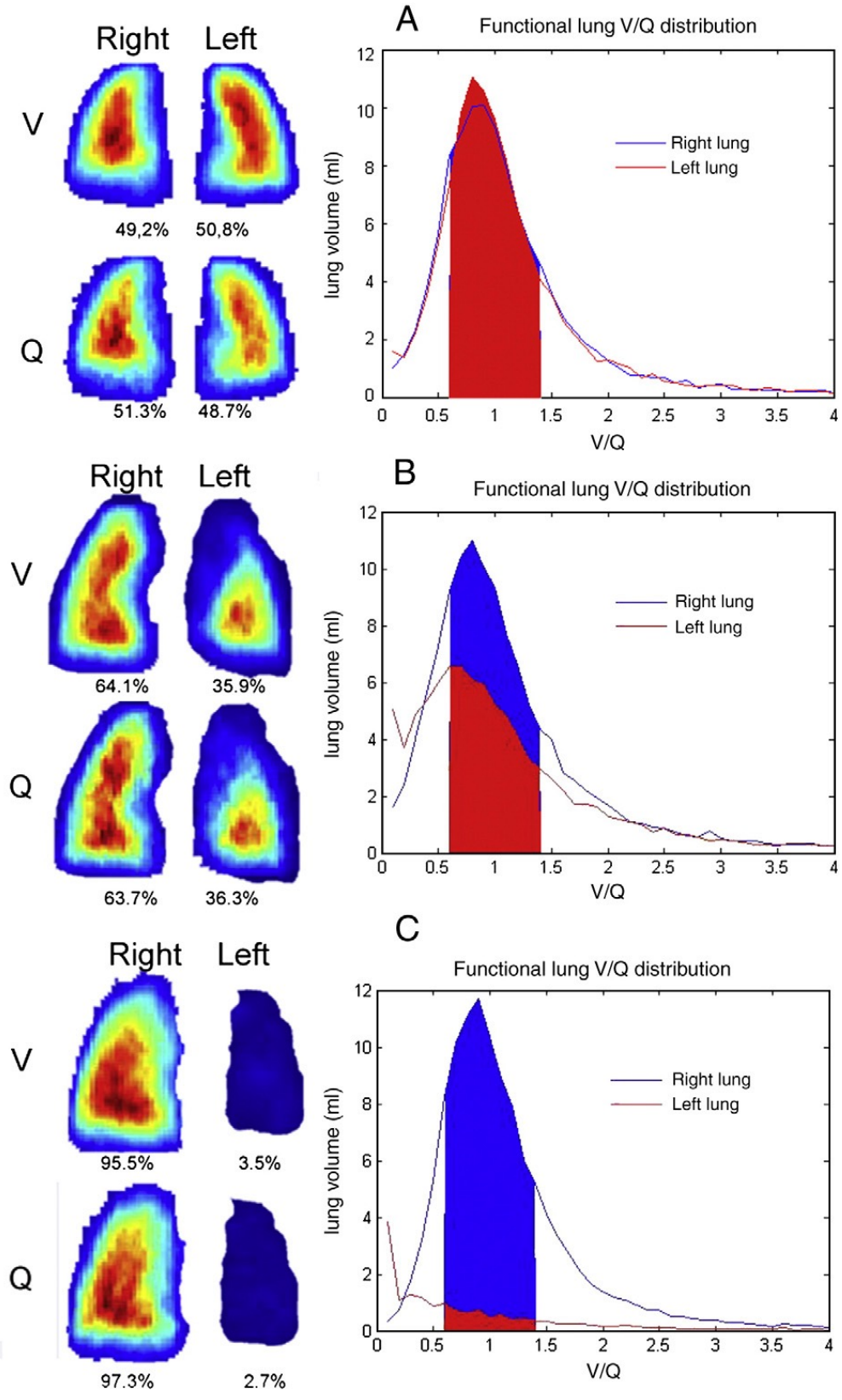


Figure 2. SPECT results for three diaphragmatic hernia patients with respectively good (A), moderate (B), and poor (C) relative ventilation (V) and perfusion (Q) distribution between the hernia side lung (left) and contralateral lung. The plots to the right represent the corresponding lung volume histograms of V/Q ratio values in each side of the lung. The colored region on the plot represents the fraction of the lung volume within V/Q [0.6, 1.4].

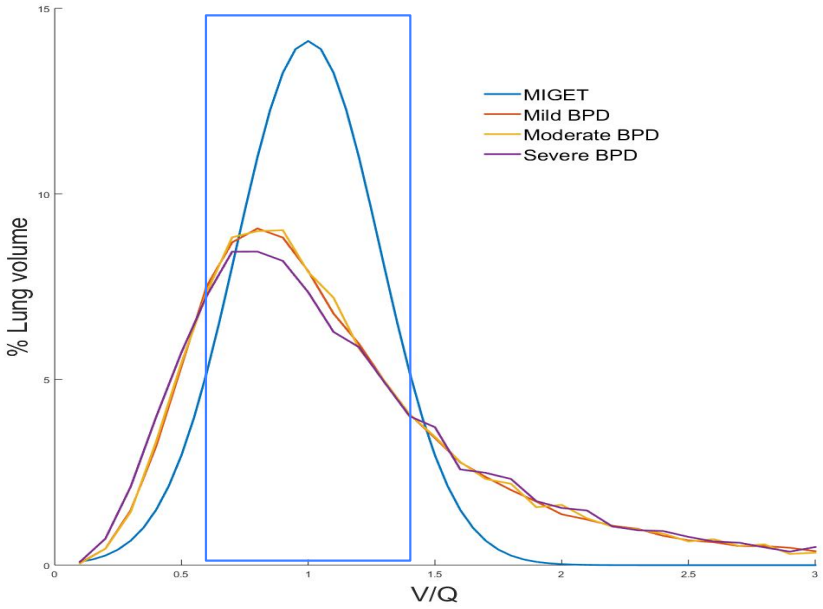


Figure 3. V/Q matching in 10-year-old children with BPD born preterm according to BPD severity group. A reference line (MIGET) indicates normal distribution of V/Q values with about 90% of the V/Q distribution within the marked box V/Q [0.6-1.4][186]. In children with BPD the distribution curves are flatter with less values within normal range and the curves are shifted to the right with larger area under the curve for V/Q>1.4 than for V/Q<0.6

4.2 SPECT AND CLINICAL GRADING/ DISEASE SEVERITY MARKER

Lung function estimated by V/Q matching is correlated to some extent with clinical severity grading. In paper I we noted that infants with an increasing severity grade of BPD had an increasing rate of V/Q abnormalities (Figure 1). However, 3/9 infants with mild BPD and 3/11 infants with moderate BPD had severe V/Q abnormalities with good matching in less than 50% of the lung. But we also noted that in the severe BPD group 4/10 had good V/Q matching within the lungs.

In study II, in the CDH patients, we noted that BPD was correlated with V/Q mismatch. Patients without BPD had better V/Q matching both in the hernia side lung and in total lung volume. Lung volume with good V/Q matching in infants with no BPD was 60% in the contralateral lung and 61% in the ipsilateral lung. In infants with BPD the corresponding values were 53% in the contralateral lung and 14% in the ipsilateral lung (Figure 4). ECMO can also be used as a marker of disease severity of CDH. Increasing days with ECMO was correlated to decreasing functional lung volume measured with SPECT in the ipsilateral lung.

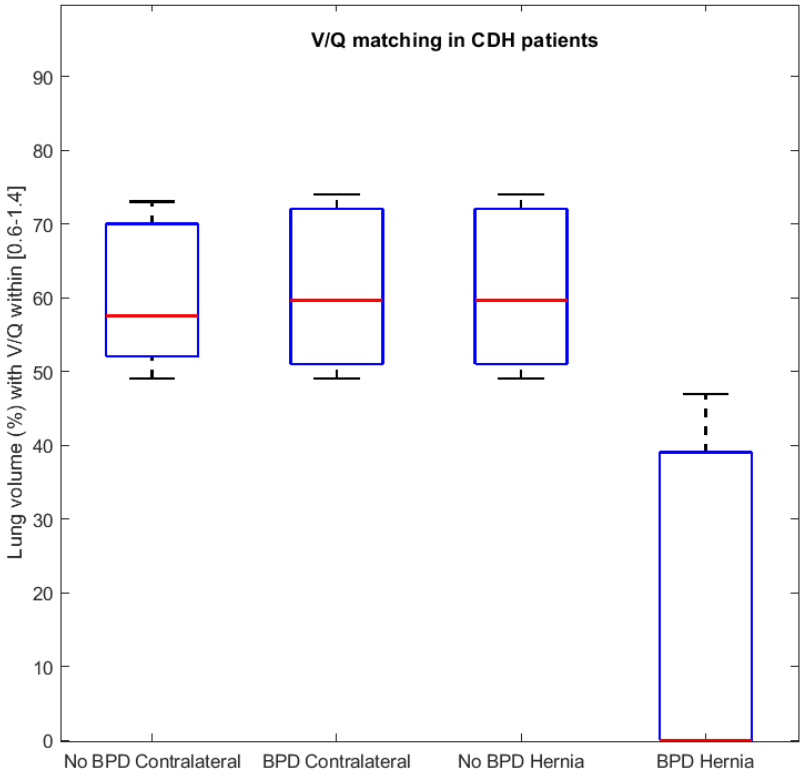


Figure 4. Lung volume with V/Q [0.6-1.4] in infants with congenital diaphragmatic hernia according to no BPD or BPD diagnosis. Contralateral side lung is shown on the left and hernia side on the right.

Contralateral side: lung volume with good V/Q matching: No BPD: 60%, BPD: 53%
Hernia side: lung volume with good V/Q matching: No BPD: 61%, BPD: 14%

In the 10-year-old children there was a trend for decreasing lung volume with good V/Q matching and increasing clinical severity grade of BPD in infancy (Figure 5). This did not reach statistical significance in our material.

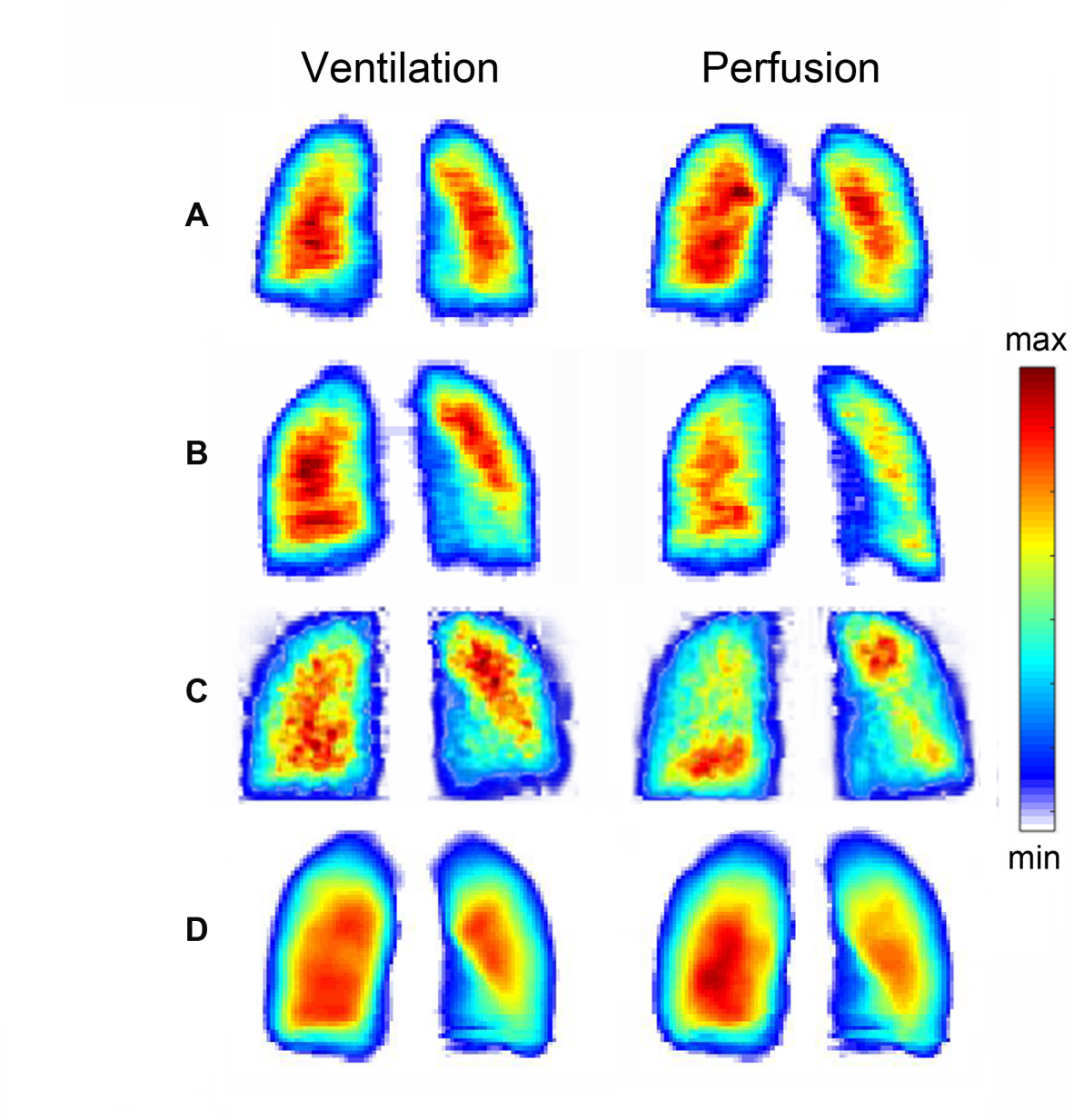


Figure 5. Representative SPECT image of three 10-year-old children with BPD clinically graded as mild (A), moderate (B) and severe BPD (C). At the bottom there is an image from a healthy individual (D).

Lung volume with good V/Q matching; **A:** 74%, **B:** 63%, **C:** 46%, **D:** 88%

4.3 CLINICAL CHARACTERISTICS AND V/Q

Presence of persistent pulmonary hypertension in the postoperative period in infants with CDH significantly correlated with the degree V and Q abnormalities.

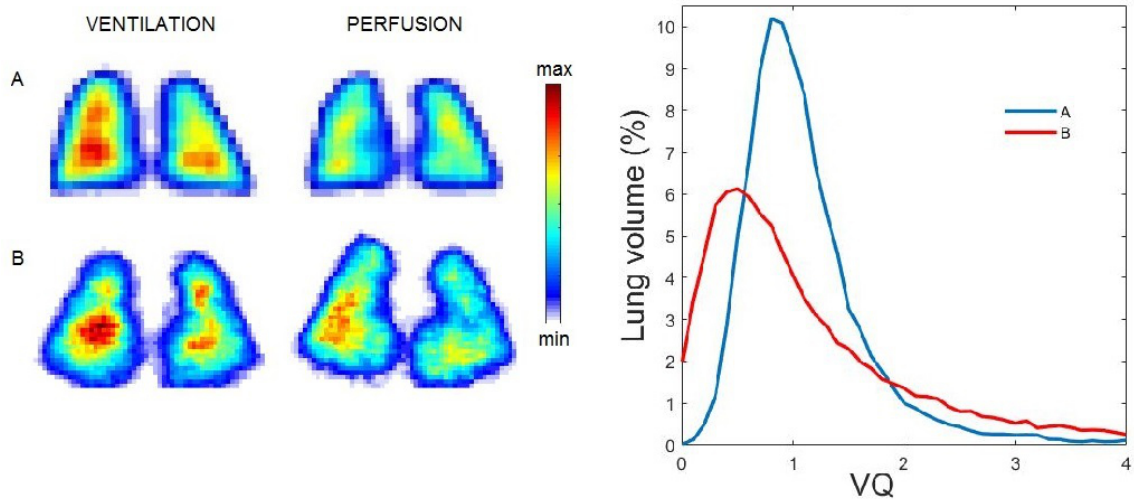
Diffusion capacity for carbon monoxide in school children with BPD had a positive linear correlation for lung volume with good V/Q matching. Other pulmonary function test were not correlated to V/Q matching.

4.4 RESPIRATORY SUPPORT AND LUNG FUNCTION

No correlation was seen in preterm infants with BPD and preterm born children with BPD between V/Q abnormalities and duration of supplemental oxygen or days with CPAP but in infants with CDH there was a correlation between longer duration of supplemental oxygen and worse function of the ipsilateral lung measured with SPECT.

In preterm infants with BPD the lung volume with satisfactory V/Q matching was decreased and in infants with CDH the ipsilateral lung volume was decreased compared to the contralateral lung volume with increasing days with mechanical ventilation. No relationship between mechanical ventilation and V/Q matching was seen in the 10-year old children.

In preterm infants with BPD we saw an association between a higher distending airway pressure on day 1 (11.9 cmH2O versus 8 cmH2O) and a larger decrease in MAP during the first week (5.6 cmH2O versus 2 cmH2O) and better V/Q matching. Higher pCO2 on day 1, (9.2 kPa (69mmHg) versus 7.3 kPa (55 mmHg)), and on average day 1-7 (8.4 kPa (63 mmHg) versus 7.5 kPa (56 mmHg)) was also associated with better V/Q matching (Figure 6, Table 1)



A: pCO2:day 1: 12.4 kPa, day 1-7: 8.98 kPa, **MAP**: day 1: 10 cmH2O, decrease day 1-7: 5 cm H2O. V/Q [0.6-1.4] 76%
B: pCO2: day 1: 6.88 kPa, day 1-7: 8.22 kPa, **MAP**: day 1: 9.0 cmH2O, increase day 1-7: 0.5 cm H2O. V/Q [0.6-1.4] 32%

Figure 6. Representative coronal SPECT slices of lung ventilation (V), perfusion (Q), and V/Q ratio distribution in preterm infants with different respiratory management. In B the V/Q curve is shifted to the left implying that ventilation is more affected than perfusion

Table 1. Mean airway pressure and pCO2 according to V/Q matching within the lung in preterm infants the first week of life

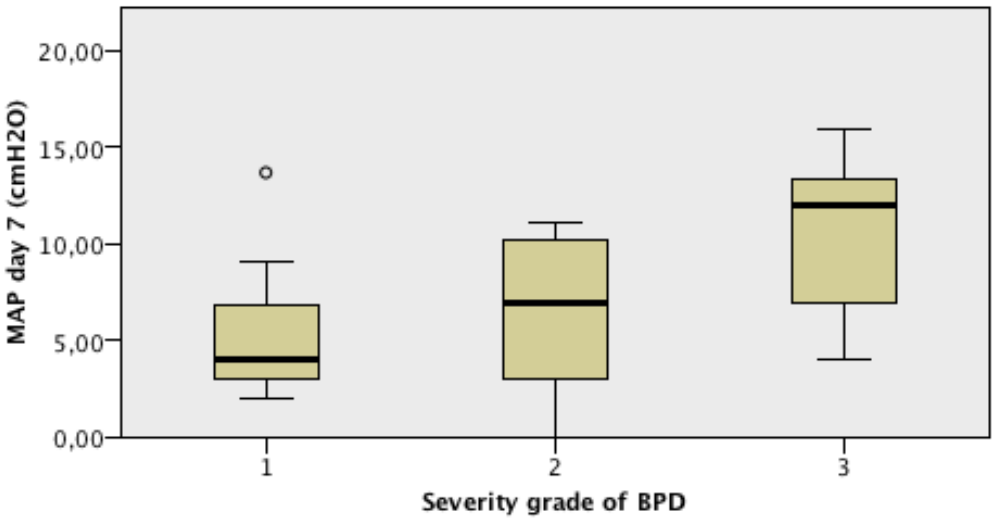
	Lung volume with V/Q [0.6-1.4] < 50% of the lung	Lung volume with V/Q [0.6-1.4] > 50% of the lung
MAP day 1 (cmH2O)	8 (4-16)	11.9 (7-21.4)*
Decrease in MAP day 1-7(cmH2O)	2 (5.1-increase 9.5)	5.6 (14-increase 3.2)*
pCO2 day 1 (kPa)	7.3 (5.6-13.9)	9.2 (6.4-21.4)*
pCO2 day 1-7 (kPa)	7.5 (5.9-9.5)	8.4 (7.1-10.1)*
Mechanical ventilation day 1	67 %	89 %
Mechanical ventilation day 7	75 %	50 %

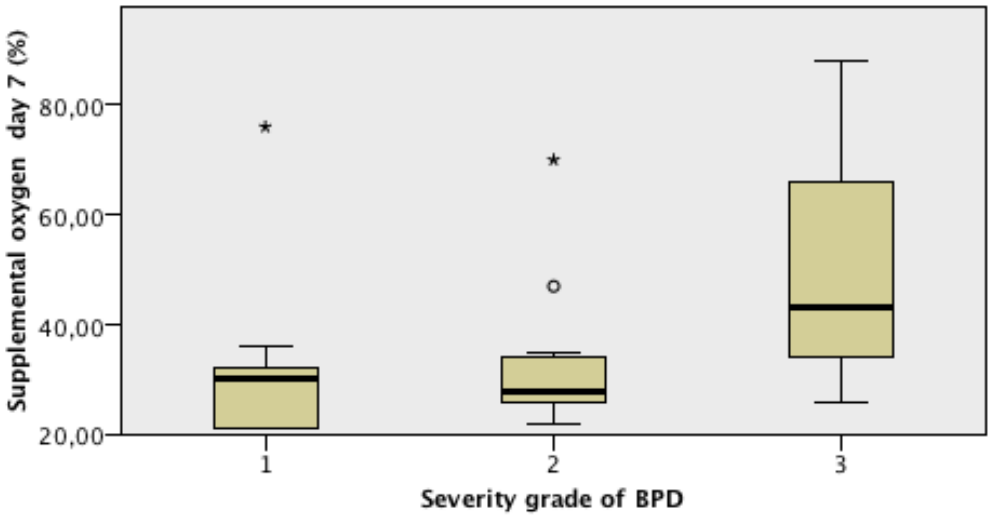
Values are median and range *p<0.05

4.5 RESPIRATORY SUPPORT AND BPD GRADING

In the preterm born infants with BPD higher mean airway pressure, oxygenation index and fraction of inspired oxygen at the end of the first week of life were associated with increasing severity grade of BPD (Figure 7). More days with mechanical ventilation, both the first week and in total, days with CPAP and supplemental oxygen also correlated with increasing severity grade of BPD (Figure 7). Preterm infants with mild BPD received surfactant at a higher postnatal age than infants with severe BPD.

In 10-year-old children increasing days with CPAP and supplemental oxygen was correlated to increasing severity grade of BPD





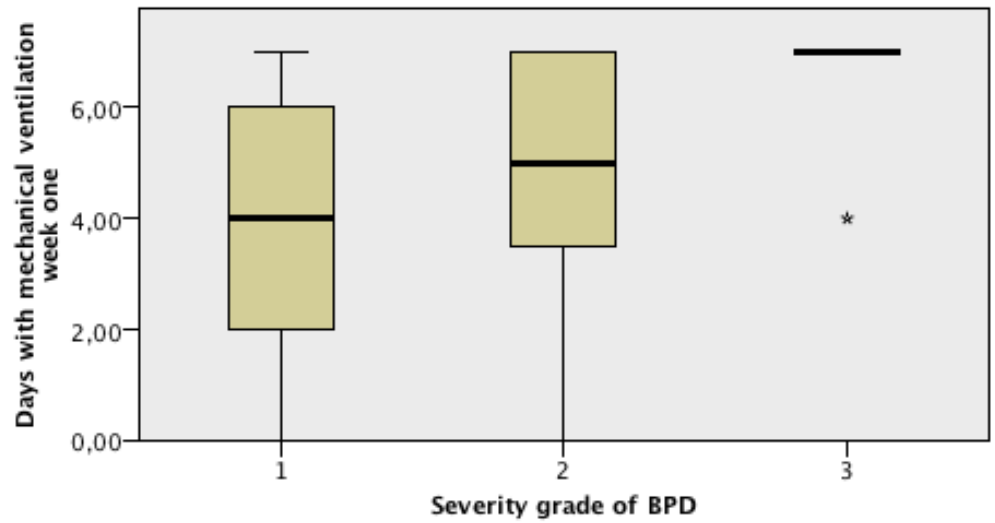


Figure 7. *Top panel:* Mean airway pressure day 7, *Middle panel*: supplemental oxygen day 7 of life, *Bottom panel*: days with mechanical ventilation first week in preterm infants according to severity grade of BPD.

* Outlier ** P<0.05 between Mild and Severe groups

4.6 CLINICAL CHARACTERISTICS AND MANAGEMENT

Mean gestational age for the preterm born infants with BPD was 26.8 week (range 23-32 weeks) and mean birth weight 976 g (range 578-1700 g). There was a significant difference in birth weight if two infants with hydrops fetalis were excluded between infants with mild and severe BPD. Infants with severe BPD received more postnatal steroids than infants with mild/ moderate BPD.

There was no association between CRIB score or respiratory index the first week (mean MAP day 1-7 x mean FiO2 day 1-7) and V/Q matching or BPD severity grade in infants born preterm with BPD.

Mean gestational age for infants with CDH was 37 weeks (range 35-41 weeks).

Mean gestational age for the preterm born10-year-old children with BPD was 26.6 weeks (range 23-32 weeks).

Lung function measurements for this group at 10 years of age have been reported earlier [210]. They had lower forced expiratory volume during 1 second, forced vital capacity and vital capacity with increasing severity grade of BPD. Diffusion capacity for carbon monoxide was in the lower range for the group as a whole but was not correlated to severity grade of BPD. There was little evidence for ongoing inflammation measured by fraction of exhaled nitric oxide. They also scored high on the Asthma control test [211], which is used to evaluate respiratory symptoms in children with asthma. I high score is representative of well-controlled asthma.

5 DISCUSSION

The survival rates of extremely preterm born infants, and infants with congenital malformations associated with pulmonary hypoplasia, have increased during the last decades. In infants born at 24 weeks gestational age, the survival rate has increased from 25% to 75% in the last 30 years [1,212]. Survival rate for infants with congenital diaphragmatic hernia has increased from 40% to 69% in the same time [10,11]. Current adult survivors with BPD, preterm born or with congenital diaphragmatic hernia, represents a different population than the children surviving today. As a group, the preterm born infants were less premature and the patients with CDH most likely had less severe pulmonary hypoplasia. Lung function results in these adult survivors may not predict the lung function in today's infants born at the limit of viability or surviving CDH infants.

Why use SPECT?

Clinical grading of BPD is a poor marker of future respiratory impairment [213]. We wanted to find a reliable measurement of lung function to complement clinical grading. This will aid us in giving more comprehensive information to parents about what to expect regarding lung function in their child after hospital discharge. SPECT is a method well tolerated in infants and children and provides unique information about regional lung function, which cannot be obtained by any other method [204]. SPECT will give a functional measurement of the lung by estimating the percentage of total lung volume with good matching between ventilation and perfusion, a prerequisite for an efficient gas exchange. SPECT in asymptomatic adult patients with normal lung function tests has revealed heterogeneities not revealed by the lung function testing and is able to sensitively predict pulmonary morbidity such as obstructive pulmonary disease [214]. The radiation dose of SPECT is also very low, only a fraction of the yearly background radiation, and significantly lower than for example computed tomography, which makes it suitable for examinations in infants and children [204]. To repeat the same functional imaging later in childhood can increase our knowledge about potential for development and repair within the lung in these children.

Summary of the principal findings

- Preterm infant with BPD, infants with CDH and preterm born children with BPD have substantial V/Q abnormalities when examined with SPECT.
- Early respiratory management was associated with V/Q matching within the lung.
- We report for the first time that a higher level of permissive hypercapnia during the first week of life may be associated with a better lung function at term.
- A higher mean airway pressure on day one and a larger-faster reduction in MAP was also associated with better V/Q matching.
- V/Q abnormalities were associated with increasing days with mechanical ventilation.

- In infants at 36-37 weeks postmenstrual age ventilation was more affected than perfusion but in children with BPD, reduced perfusion contributed more to the V/Q abnormalities.
- The NIH definition of BPD created in 2001 based on oxygen requirement is correlated to V/Q mismatch in preterm infants and infants with CDH. However it did not identify some patients with severe V/Q abnormalities and defined other patients with good V/Q matching within the lungs as severe BPD.
- Some children, in the 10-year-old group, with good values on lung function testing who did not report any respiratory problems or problems in participation in physical activities had widespread V/Q abnormalities.
- Respiratory requirement at one week of age and days with mechanical ventilation the first week of life and in total were correlated to BPD severity grade.
- Total days with supplemental oxygen was not correlated to V/Q mismatch in preterm subjects with BPD.

Importance and relevance of our V/Q findings

Infants with BPD, infants with CDH and children with BPD born preterm have substantial V/Q abnormalities. In preterm infants with BPD only 54% of the total lung volume had satisfactory V/Q matching. In infants with CDH both lungs were affected. At the same side as the hernia, 38% of the lung volume had good V/Q matching, and on the other side this figure was 56%. Hence, infants with CDH born at a higher gestational age (10 weeks on average) had worse lung function measured with SPECT than extremely/ very preterm born infants. In some patients with CDH the ipsilateral lung hardly contributed to gas exchange at all making these patient completely dependent on one lung. In children, diagnosed with BPD in infancy, 62 % of the lung volume had satisfactory V/Q matching, which is still lower than in lung healthy children at the same age. According to the literature, lung volume with good V/Q matching should be around 89% [205,206,209,215].

What is unique with our studies is that they, for the first time, demonstrate the presence of V/Q defects in infants with BPD and CDH and that the V/Q abnormalities persist in survivors with BPD. They demonstrate a functional loss in the lung that has significance for both short and long term health in these patients.

Association of V/Q to early respiratory management

Respiratory strategies and requirements were associated with V/Q abnormalities at term age. Hypocapnea is a known risk factor for BPD [107,108]. Hypercapnia has in some studies been associated with increasing rates of moderate/ severe BPD [114]. Other studies indicate that hypercapnia might be an indicator of disease severity and not a risk factor per se[115].

We are the first ones to show that higher levels of permissive hypercapnia on the first day of life and the first week of life might be associated with a better lung function. Higher values of

pCO2 were associated with less V/Q abnormalities at term age. Respiratory index was not correlated to V/Q matching or clinical grade of BPD.

An open lung strategy with higher MAP to open up the lung in the beginning but early weaning of mechanical ventilation was also associated with better V/Q matching. Other studies support that early extubation is lung protective even if reintubation is needed [105,106]. However, it seems safe to wait with surfactant since in our study infants with mild BPD received surfactant at a later postnatal age than infants with severe BPD. This is also in line with the published literature [216]. Total number of days with supplemental oxygen is a poor marker for respiratory morbidity according to the literature. Our finding of no correlation between total days with supplemental oxygen and V/Q matching agrees with this.

Repair of the lung?

We find it promising that the lung volume with good V/Q matching seems to be higher in BPD children than in preterm infants with BPD. The range of lung volume with good V/Q matching was also narrower, 46%-82% in children with BPD compared to 28%-76% in preterm born infants with BPD. Hence, there were no individuals with extremely low matching between ventilation and perfusion among the children but this was more common among the infants. This could possibly point in a direction of a potential for regeneration and repair even in the worst cases. This is also supported by the fact that V/Q abnormalities were correlated with increasing days with mechanical ventilation in infants but not in children. The initiation of ventilation either manual or mechanical is a major risk factor for developing BPD [47,79]. We speculate that the acute effects of volutrauma from MV are mitigated over time with the postnatal reparative process and continued alveolarization. We cannot be certain of these findings, because although similar in gestational age, the patients came from different cohorts. A longitudinal study in one set of patients would be of value.

Ventilation or perfusion abnormalities?

In preterm infants with BPD we saw a left shift of the V/Q distribution with increasing severity grade of BPD thus indicating that ventilation was more affected than perfusion while we saw the opposite in the 10-year-old children and some of the infants with CDH with the perfusion more affected than the ventilation. The preterm infants with BPD were examined at an earlier age than the infants with CDH (37 weeks postmenstrual age and 6 months corrected age respectively). We speculate that the preterm infants with BPD are still in the acute phase with ongoing inflammation and airway obstruction while the infants with CDH and the preterm children with BPD are in a more chronic phase of lung disease.

Evidence of new and old BPD

The visual appearance of the SPECT scans reveals a heterogeneous pattern of V/Q defects increasing in number with the increasing severity grade of BPD giving a patchy impression of scattered areas with poor V/Q matching. In the description of the histopathology of the “new” BPD, seen in the surfactant era, a more uniform disease is observed. It describes an arrested

alveolar development, including both airspaces and vasculature resulting in a simplification of the distal structure and a reduced diffusion surface area [26]. This is in marked contrast to the descriptions of "old" BPD in the pre-surfactant era with a severely damaged lung with variously atelectatic or enlarged air spaces and an almost cobblestone like appearance. Are we seeing structural defects resembling old BPD reflected in the patchy distribution of the most prominent V/Q defects? An uneven surfactant distribution will often exist in more severe cases and this will lead to areas more susceptible to volutrauma and uneven injury patterns The nature of the injury could play a role as volutrauma in the surfactant deficient lung has been shown to be a major risk factor in the acute lung injury caused by ventilation [217]. A combined anatomic and functional investigation using CT-SPECT would be one way of looking at these areas more closely

Potential for development and repair

There is some evidence that alveolarization might have a greater potential for recovery than the abnormal and reduced vascularization seen in preterm born subjects with BPD [5,167,218,219]. This has also been demonstrated in subjects with CDH. Vascular growth does not, in some patients, match alveolar development after the acute phase. Follow up studies to five years of age show that in infants with CDH ventilation is normalized to a greater extent but perfusion remains affected [195]. Early disturbances in vascularization might not have the same potential for recovery. This might also be true for preterm children with BPD with the findings of a more reduced perfusion in our study of the 10 year-old BPD children.

Clinical grading of BPD in relation to V/Q findings

A concern is that the clinical oxygen and/or positive pressure criteria at 36 weeks may not identify important pulmonary abnormalities that can lead to a reduced lung capacity in BPD survivors [33,220]. We found that in patients with mild BPD 33% had V/Q mismatch in more than 50% of the lung indicating a risk for a reduced pulmonary function in the future. The implication of this finding is that the severity grading used at the time of our study may miss patients with significantly reduced lung function and an ensuing risk that these patients may miss out on closer longitudinal follow-up of their lung function. The definition based on oxygen requirement and respiratory support has its merits though. It is easy to apply and has some predictive value.

A refinement has been suggested for the definition and grading of BPD [36]. It is also based on oxygen requirement and need for respiratory support but also includes newer modes of respiratory support like high flow nasal cannula. It states that a premature infant with BPD should have radiographic confirmation of parenchymal lung disease without specifying what these findings should be.

In infants with BPD we found that higher mean airway pressure, oxygenation index and higher oxygen requirement were associated with increasing severity of BPD. One week of

age might be a good time point to identify patients who are candidates for more alternative treatment or therapeutic strategies in research settings.

Severity markers for congenital diaphragmatic hernia

Presence of persistent pulmonary hypertension in the postoperative period in infants with CDH is strongly correlated with V and Q abnormalities. This is in line with the literature suggesting that persistent pulmonary hypertension is related to the degree of vascular hypoplasia within the lung in CDH patients. If the vascular hypoplasia is pronounced, the lung cannot accept the right ventricular output with a poor outcome as a result [9]. Persistence of PPHN post-surgery is associated with decreased survival. In one report the mortality was 100% if PPHN was sustained at 6 weeks of age [221].

Increasing days with ECMO was also associated with V/Q abnormalities. The need for ECMO is a known risk for poor outcome. Survival for infants not requiring ECMO is 70-90% while it is 50% in infants who do [222]. A diagnosis of BPD was also correlated to increasing V/Q abnormalities. V and Q abnormalities seem to be important elements in the pathophysiology of CDH.

Long-term follow-up

Pulmonary function in children diagnosed with BPD in infancy is often evaluated by lung function testing or reported symptoms only. The group of 10-year-old children with BPD had low dynamic flow values. They had lower forced expiratory volume during 1 second, forced vital capacity and vital capacity with increasing severity grade of BPD and on average a lower diffusion capacity for carbon monoxide (DLCO) than normal. This agrees with findings showing longitudinal limitations in airflow in subjects with BPD and in preterm infants generally [162,223]. Studies on infants with developing BPD in infancy have reported abnormalities in measured variables, especially compliance and lung volumes that correlate with the severity of BPD [224,225]. With increasing age, the pattern of lung function abnormalities on traditional PFT in survivors with BPD seem to mostly reveal airflow limitations, while mechanics and volumes improve perhaps due to increasing alveolarization. In our patients the dynamic flow values were not correlated to V/Q, but we see that higher DLCO was correlated to better V/Q matching but not BPD grade. DLCO is partly a measurement of V/Q matching and function of the alveolar-capillary unit. DLCO was in the lower range for the group, thus confirming the residual abnormalities in lung function.

Some of the children had relatively normal lung function testing values but abundant V/Q abnormalities. With SPECT you can discover functional abnormalities not detected by lung function testing [214,226] In general the children diagnosed with BPD in infancy scored high on Asthma control test, indicating few respiratory symptoms despite low values on dynamic spirometry and a reduced lung function measured with SPECT.

Some well appearing infants with CDH and no respiratory symptoms proved to have practically no lung function in on the hernia side and 10-year-old children with a history of BPD who reported no limitation in exercise capacity or respiratory symptoms proved to have substantially lower functional lung volume than lung healthy children the same age. We speculate that children and their parents are not aware of these abnormalities but have just adapted their life style accordingly. These children would probably benefit from early physical training to increase their lung capacity.

Methodological limitations

We did not perform a simultaneous CT scan; therefore we could not outline the borders of the lung anatomically, only functionally. Thus, we may have missed areas at the outer borders of the lung with matched V/Q defects and underestimated V/Q abnormalities.

Even though the radiation dose for SPECT is low, a fraction of the yearly background radiation dose, we could not recruit an age matched control group for reference. This is due to ethical restrictions that were imposed by the radiation protection and ethics committees of Stockholm County.

No reference values exist for V/Q SPECT in infants and children. We had to compare our results with healthy subjects examined at our department (children and adults), reference values from the literature on V/Q matching and children examined with MIGET (the golden standard for V/Q). Previous work shows that V/Q ratios measured with the SPECT technique are in good agreement with MIGET (r2=0,99) [209]. In children with CDH we compared the ipsilateral lung with the contralateral lung and risk underestimating the abnormalities in the ipsilateral lung since the contralateral lung can also be affected.

Further our sample size is small, and this introduces a risk of missing some significant differences.

6 CONCLUSION AND REFLECTIONS

SPECT can be used as a measurement of lung function and evaluation of treatment in neonatal patients, infants and children. It can also be used as a complement to clinical grading and for follow-up of lung function in survivors with BPD. Ventilation perfusion matching abnormalities are common among patients with BPD both in the newborn period and at school age suggesting the presence of residual alveolar-capillary impairment.

The current definition does not identify all individuals with pulmonary abnormalities and should be revised to include a functional measurement of the lung.

Since extreme preterm born infants as a group have a reduced pulmonary function we recommend that all extremely preterm born infants, even subjects without BPD, should have an evaluation of lung function before discharge to identify patients with abnormalities and include these in pulmonary follow-up programs.

We must strive to refine our early management of extremely preterm infants. Respiratory management the first day of life and the first week have associations with V/Q matching at term age. Higher values of pCO2 and an open lung strategy the first day of life with early weaning of mechanical ventilation were associated with better V/Q matching at term age.

Respiratory requirement at one week of age was correlated with BPD severity grade at 36 weeks postmenstrual age. Infants at risk at 7 days of age should be considered for inclusion in research studies on treatment alternatives.

The criteria for CPAP treatment placing an infant in the severe BPD category can be questioned since 40% of infants with severe BPD in our study, who all had CPAP at 36 PMA, had good V/Q matching within the lung. We welcome the refinement of grading by Higgins, 2018, where infants with CPAP and FiO2 <0.3 are graded as BPD I or II.

The criteria of including radiographic findings of parenchymal lung disease at 36 PMA in the Higgins definition can also be questioned since infants with new BPD can have arrested alveolar development and reduced vascularization without radiographic abnormalities and we instead suggest a functional imaging to get a better evaluation of actual lung function.

7 FUTURE PERSPECTIVES

The advances in functional pulmonary imaging have opened up new possibilities of understanding the pathophysiology of lung function impairments in prematurely born infants. The diagnosis of bronchopulmonary dysplasia is based on the treatment of the disease, a requirement of supplemental oxygen and or respiratory support at a certain age. Within this group all kinds of different lung function abnormalities coexist. “Old” BPD with mechanical injuries, “new” BPD with arrested alveolar development and decreased vascularization, lung hypoplasia due to congenital abnormalities or infants small for gestational age, lung hypoplasia due to anhydramnios of different causes and genetically more susceptible infants. The lung function abnormalities will also depend on when in the developmental phase of the lung they occurred and what iatrogenic factors the infant is exposed to. What we know is that all extreme premature infants are at risk for adverse pulmonary outcome as are many of the more mature infants with other risk factors.

All infants with risk factors for adverse pulmonary outcome should be followed longitudinally with measurements of lung function.

Oxygen requirement at 36 weeks postmenstrual age or 56 days is a poor marker for lung function. Pulmonary functional testing does not fully evaluate distal pulmonary function and

gas exchange and is not sensitive to subtle changes in the alveolar-capillary unit. Anatomical imaging can give some information but does not evaluate actual lung function. SPECT in the newborn period, during childhood and at young adult age could enhance our understanding of the pathophysiology of the developing lung in these patients. How widespread are the ventilation and perfusion abnormalities? What is the potential for catch-up of developing functional alveolar-capillary units? Do the arrested alveolar development and the abnormal vascularization have the same potential for normalization? Is the mechanism similar between preterm infants with BPD and infants with congenital diaphragmatic hernia both with evidence of vascular hypoplasia on histological examination?

Combining SPECT with CT in selected cases can increase resolution and correlate radiographic findings with lung function impairments to further enhance our understanding of these abnormalities.

Further development of functional MRI to make it clinically available would be desirable as radiation could then be avoided completely.

Evaluation of early treatment strategies and new therapies should be performed with a functional measurement and not just oxygen requirement at 36 weeks postmenstrual age since this gives limited information about actual lung function.

Hopefully a new definition and grading for BPD can be developed in the near future including a functional measurement to predict future lung impairment. This will enable us to benchmark centers with different treatment strategies and evaluate new therapies.

8 SWEDISH SUMMARY

Överlevnaden för extremt förtidigt födda barn och barn med medfödda missbildningar har ökat kraftigt de senaste 30 åren. Många av dessa barn får en nedsatt lungfunktion. Att ta hand om dessa individer på bästa sätt är ett stort ansvar för samhället. De ska inte bara överleva tiden på sjukhus utan även få en bra barndom och fungera normalt i vuxenlivet. Min avhandling har främst fokuserat på hur vi kan utvärdera dessa barns lungfunktion.

Kronisk lungsjukdom hos spädbarn, även kallat bronchopulmonell dysplasi (BPD) (bronk=luftväg, pulm=lunga, dysplasi=sjuklig förändring i vävnad ofta som svar på yttre retning) beskrevs redan för 50 år sedan av William Northway. Han konstaterade att måttligt förtidigt födda barn, som avlidit på grund av andningssvikt, hade kraftiga avvikelser i hur lungvävnaden såg ut. Vissa delar av lungan var sammanfallna och andra delar alltför uppblåsta. Vissa lungblåsor, alveolerna, var helt stängda och vätskefyllda och andra hade spruckit upp. Lungvävnaden var kraftigt inflammerad med ärrbildning och avvikelser i lungblodkärlen. Orsaken till dessa skador var troligen respiratorbehandling med höga tryck som gav mekaniska skador och höga nivåer av syrgas som i sig kan ge en inflammatorisk reaktion. Under 90-talet skedde ett flertal genombrott inom neonatalvården som ledde till att fler mycket förtidigt födda barn överlevde. Dessa barn hade också ett långvarigt syrgasbehov men den mikroskopiska bilden i lungvävnaden såg annorlunda ut med mindre inflammation och mekaniska skador. Man noterade istället att den utveckling av alveoler och blodkärl som normalt sker den sista tredjedelen av graviditeten uteblivit eller försenats.

Den definitionen för BPD som används mest idag är att barnet fortfarande behöver extra syrgas vid 28 dagars ålder. Svårighetsgradering sker då hos mycket förtidigt födda barn i motsvarande vecka 36 och annars vid 56 dagars ålder beroende på behov av extra syrgas eller annat andningsunderstöd. Vissa använder bara syrgasbehov/ behov av andningsunderstöd vecka 36 som definition för BPD. Bägge dessa definition har dock många brister. Behov av extra syrgasbehov eller andningsunderstöd behöver inte betyda att barnet har en underliggande lungsjukdom och alla barn med nedsatt lungfunktion kräver inte extra syrgas. Nästan alla barn födda före den 28:e graviditetsveckan har syrgas eller andningsstöd vid 28 dagars ålder. Detta kan bero på ett omoget andningsmönster eller en ännu inte fullt utvecklad lunga och behöver inte betyda att barnet kommer att få en framtida lungfunktionsnedsättning. Även vecka 36 kan syrgasbehovet bero på andra faktorer såsom magbesvär, omogenhet, infektion eller annan tillfällig försämring. Med dessa definitioner riskerar man att missa vissa barn med kroniskt nedsatt lungfunktion och felaktigt identifiera andra som sjuka.

Syftet med min avhandling var att försöka komplettera den definition som används för BPD idag med en funktionell undersökning av lungan för att kunna ge en prognos om risk för framtida lungbesvär, planera uppföljning och behandling samt identifiera eventuellt behov av speciellt inriktade träningsinsatser. Den funktionella lungundersökningen kan även användas för att utvärdera effekten av olika behandlingsstrategier och följa utvecklingen av lungan under barndomen och upp i vuxen ålder.

Det är komplicerat att undersöka lungfunktion hos spädbarn. De flesta röntgenmetoder ger bara en anatomisk bild av lungan utan någon information om faktisk funktion. Spirometri i nyföddhetsperioden är undersökarberoende och svårt att genomföra på de sjukaste patienterna och man kan därför sällan dra några generella slutsatser. Spirometri undersöker dessutom framförallt de medelstora och små luftvägarna. Hos barn med BPD är det mestadels de distala delarna av lungan, alveoler och lungkapillärer, som är drabbade,

Lungans viktigaste funktion är att ta upp syre i kroppen och göra sig av med koldioxid. För att detta ska kunna ske så effektivt som möjligt behöver man en stor yta för diffusion av syre från alveolen in i kapillärerna och en lika stor yta för diffusion av koldioxid från blodet till den luftfyllda alveolen. En försämrad lungfunktion kan bero på stopp i de stora luftvägarna genom till exempel svullnad i svalget, svullnad av de små luftvägarna vid till exempel astma eller ett ökat avstånd mellan alveolar och kapillärer vid till exempel mekaniska eller inflammatoriska retningar. Den alveolära ytan kan också vara minskad till följd av emfysem eller avstannad/försenad utveckling vid BPD. Blodkärlen kan ha förtjockade väggar eller vara underutvecklade till följd av medfött diafragmabråck som har komprimerat lungvävnaden. Luften och blodet kan också ha en dålig matchning i lungan och inte gå till samma ställen. Hos förtidigt födda barn med BPD är det ofta en kombination av flera orsaker som leder till lungfunktionsnedsättningen men framförallt är det en störning i gasutbytet mellan alveoler och kapillärer. För att få en uppskattning av lungans förmåga till ett effektivt gasutbyte kan man undersöka hur blodet och luften fördelar sig i lungan i förhållande till varandra, om det är ”matchat”. Single photon emission computed tomography (SPECT) är en teknik för att undersöka fördelning av ventilation och blodflöde i lungan tredimensionellt. Tidigare har man inte kunnat göra denna undersökning på spädbarn. Genom teknisk utveckling, delvis utförd i vår forskargrupp, är det nu möjligt. Vi har undersökt 30 förtidigt födda barn med BPD i nyföddhetsperioden (studie I och IV), 12 barn med medfött diafragmabråck vid cirka 6 månaders ålder (studie II) och 26 förtidigt födda barn med BPD som nu är cirka 10 år gamla (studie III) med SPECT. Vi har även tittat på behandling och sjuklighet under vårdtiden, svårighetsgrad av BPD och hos de 10 åriga barnen, resultat på lungfunktionsmätning med spirometri.

Vi kunde konstatera att de flesta barnen hade omfattande avvikelser i matchning mellan luft och blodflöde i lungan. Dessa avvikelser överensstämde till viss del med svårighetsgraderingen av BPD. Vissa barn som fått diagnosen svår BPD hade en i stort sett normal matchning av luft och blodflöde i lungan. Andra som fått diagnosen mild BPD eller inte fått någon BPD diagnos hade grava avvikelser i lungfunktion mätt med SPECT. Vi noterade också att vissa barn i 10 års ålder som själva inte upplevde sig ha några lungbesvär eller fysiska begränsningar på grund av nedsatt lungfunktion och hade normala värden på spirometri hade omfattande avvikelser i matchning av luft och blod i lungan. Dessa barn har en hög risk för framtida lungfunktionsnedsättning och kan ha nytta av en tät uppföljning och tidiga insatser vid eventuell försämring.

Vi noterade också att ju fler dagar barnet haft i respiratorbehandling, desto sämre var lungfunktionen mätt med SPECT. Även i andra studier har man kunnat konstatera att förtidigt födda som behandlas med CPAP istället för respirator löper mindre risk att utveckla BPD. Våra fynd tyder på att vi bör sträva efter att minska antalet dagar med respiratorbehandling och försöka extubera förtidigt födda barn och barn med medfött diafragmabråck så tidigt som möjligt. Vi konstaterade också att förtidigt födda spädbarn som haft högre koldioxidvärden i blodet första veckan, så kallad ”permissiv hyperkapne”, hade bättre matchning av luft och blodflöde i lungan. Man har tidigare visat att onormalt låga värden av koldioxid ökar risken för BPD men man har inte kunnat visa att höga jämfört med normala värden minskar risken för BPD. Genom att tillåta högre koldioxidvärden kan man minimera tryck i respirator och extubera tidigare.

Vi anser att alla extremt förtidigt födda spädbarn och spädbarn med riskfaktorer för lungfunktionsnedsättning bör göra en funktionell undersökning av del av lungan som kan förväntas vara påverkad. Detta för att tidigt identifiera barn i riskzonen. Ur ett samhällsperspektiv kan man även spara resurser genom att tidigt sätta in behandling vid försämring och därmed minska risken för återinläggningar på sjukhus vid till exempel luftvägsinfektioner och ge specifika tränings råd till de barn som behöver för att främja deras lungkapacitet i framtiden.

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