# BRONCHIAL LABILITY IN SCHOOLCHILDREN BORN VERY PRETERM

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Helsinki 2000

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Academic dissertation

To be publicly discussed by permission of the Medical Faculty of the University of

Helsinki, in the auditorium of the Hospital for Skin and Allergic Diseases, Meilahdentie 2, Helsinki, on January 21st, 2000, at 12 noon.

Helsinki 2000

ISBN 951-45-8993-9 (PDF version)
Helsinki 2000
Helsingin yliopiston verkkojulkaisut

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## LIST OF ORIGINAL PUBLICATIONS

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I. Pelkonen AS, Hakulinen AL, Turpeinen M. Bronchial lability and responsiveness in schoolchildren born very preterm. Am J Respir Crit Care Med 1997; 156:1178-1184.

II. Pelkonen AS, Hakulinen AL, Turpeinen M, Hallman M. Effect of neonatal surfactant therapy on lung function at school age in children born very preterm. Pediatr Pulmonol 1998; 25:182-190.

III. Pelkonen AS, Suomalainen H, Hallman M, Turpeinen M. Peripheral blood lymphocyte subpopulations in schoolchildren born very preterm. Arch Dis Child Fetal Neonatal Ed 1999; 81:F188-F193.

IV. Pelkonen AS, Hakulinen AL, Hallman M, Turpeinen M. Effect of inhaled budesonide therapy on lung function in schoolchildren born very preterm. Submitted.

V. Pelkonen AS, Nikander K, Turpeinen M. Reproducibility of home spirometry in children with newly diagnosed asthma. Pediatr Pulmonol in press.

**ABBREVIATIONS**

| | |
|---|---|
| AGA | Appropriate for gestational age |
| ATS | American Thoracic Society |
| BAL | Bronchoalveolar lavage |
| BHR | Bronchial hyperreactivity |
| BPD | Bronchopulmonary dysplasia |
| $C_L$ | Lung compliance |
| $C_{LCO}$ | Diffusing capacity of the lung |
| CD | Cluster of differentiation |
| CLD | Chronic lung disease |
| COPD | Chronic obstructive pulmonary disease |
| $D_{LCO}$ | Diffucing capacity of the lung |
| $D_L/V_A$ | Specific diffucing capacity |
| ECP | Eosinophil cationic protein |
| $FEV_1$ | Forced expiratory volume in one second |
| $FEF_{50-75}$ | Forced expiratory flow at 50-75% of vital capacity |
| $FiO_2$ | Fraction of inspired oxygen |
| FRC | Functional residual capacity of the lung |
| FVC | Forced vital capacity |
| GC | Glucocorticoid |
| IMV | Intermittent mandatory ventilation |
| IUGR | Intrauterine growth retardation |
| MPO | Myeloperoxidase |
| NK cells | Natural killer cells |
| $PD_{15}FEV_1$ | Histamine dose (mg) resulting in a 15% decrease in $FEV_1$ |
| PDA | Patent ductus arteriosus |
| PEF | Peak expiratory flow |
| $PIF_{TBH}$ | Peak inspiratory flow through Turbuhaler |
| $R_{aw}$ | Airway resistance |
| RDS | Neonatal respiratory distress syndrome |

| | |
|---|---|
| RV | Residual volume of the lung |
| $SG_{aw}$ | Specific airway conductance |
| SP-A/DPC ratio | Surfactant protein A/disaturated phosphatidylcholine ratio |
| TLC | Total lung capacity |
| VDSS | Vitalograph Data Storage Spirometer |
| VLBW | Very low birth weight (500-1500g) |

## 1. INTRODUCTION

The long-term follow-up of preterm infants with or without diagnosed chronic lung disease (CLD) is an ongoing saga. The history of this chronic lung disorder is relatively young, having been described a little more than 30 years ago (Northway et al 1967). When Northway et al (1990) reported a long-term follow-up of infants with CLD, the results were surprisingly good, indicating mild bronchial obstruction and increased bronchial responsiveness. These findings have been confirmed by other studies. Bronchial obstruction, hyperresponsiveness with mild respiratory symptoms regardless of CLD are common in schoolchildren born preterm (Gross et al 1998). However, the results of these long-term studies on a cohort of children inform us about the effect of a particular therapeutic approach that may have completely changed by the time the study is completed. During the 1970s, continuous positive airway pressure and positive end expiratory pressure were introduced. In the late 1980s, surfactant replacement therapy again changed the population of surviving infants. The influence of this therapy on the long-term prognosis has not been studied. Antenatal and prenatal steroid therapy may also have an effect on long-term lung function. Today, infants born after 30-32 weeks of gestation and ventilated for respiratory disease only exceptional die or develop CLD. The average surviving preterm baby with CLD is therefore more premature than previously. This may lead to increased severity of pulmonary disease and a poorer outcome in spite of the rapid advances in neonatal care.

In schoochildren born preterm the causes of abnormal lung function are complex. Impaired airway growth in infancy, smooth muscle hypertrophy, peribronchial and bronchial fibrosis, and pathological changes in the bronchial mucosa have been suggested (Marcgraft et al 1991, Chan et al 1993). Neutrophilic inflammation plays a prominent role in the pathophysiology of early CLD in preterm infants (Merritt et al 1983, Yoder et al 1991). It is not known whether chronic inflammation plays a role in the increased bronchial responsiveness of schoolchildren born very preterm, as it does in asthma or whether these changes are responsive to treatment. The beneficial effect of glucocorticoids (GC) on lung disease has been demonstrated in ventilator-dependent neonates (Yoder et al 1991, Bhuta and Ohlsson 1998, Cole et al 1999) and in some wheezy preterm infants (Yuksel et al 1992b). There has been one report of short-

term treatment with inhaled GC at school age. This treatment did not influence basic lung function or bronchial responsiveness in children born preterm (Chan et al 1993).

With the aim of evaluating basic lung function, respiratory symptoms, bronchial lability and bronchial responsiveness in every-day life in schoolchildren born very preterm and any findings suggesting inflammatory lung disease, we studied a total of 63 schoolchildren born very preterm. Our aim was also to investigate the lung function of a cohort of children who, as small preterm infants, participated in randomized trials of surfactant therapy in order to assess the effect of surfactant. In order to evaluate bronchial lability in these children, we used a novel data storage home spirometer.

# 2. REVIEW OF THE LITERATURE

## 2.1. Lung function during infancy in children born preterm

### 2.1.1. The premature lung and respiratory distress syndrome

The lungs of preterm infants are both structurally and functionally immature. With premature birth, normal lung growth and development are disrupted and the immature lung is exposed to adverse stimuli at the time when it is most susceptible to injury related to oxidants, barotrauma and/or infection-inflammation. Lung structural development may be divided into the following five phases (Langston et al 1984). 1. The embryonic period, formation of proximal airways, bronchi, (4 to 6 weeks) 2. The glandular period, formation of conducting airways, bronchioles, (7 to 16 weeks), 3. The canalicular period, formation of acini, the structural development of the alveolar-blood barrier, (17 to 27 weeks), 4. The saccular period, expansion of the gas-exchange units, primitive alveoli, (28 to 35 weeks), and 5. The alveolar period: expansion of surface area due to formation of true alveoli (36 to 3 years post-term). Functionally during foetal life, gas-exchange is performed by the placenta and a fluid fills the developing future airspaces until birth. Surfactant synthesis in type II alveolar cells begins to increase at 20 weeks of gestation (Hansen and Corbet 1998). In infants of early gestational age, there is clearly a difference in the maturational level of the lung. Lung maturation does not depend only on gestational age. Genetic factors and several pharmacological effectors (prolactin, glucocorticoids, sex hormones, insulin and catecholamines) associated with complications of pregnancy modify the normal timing of lung maturation (Merkus et al 1996). Collagen is the major connective tissue element of non-respiratory component of lung. Elastin predominates in the gas-exchanging lung parenchyma. In newborn infants, elastic recoil is low because of sparse elastic fibres. After birth, the elastic content of the elastin fibres increases rapidly to reach adult levels by 6 months of age (Keeley et al 1977). Impaired respiratory mechanics including an elastic rib cage, insufficient connective tissue of conductive airways and incomplete development of the terminal air spaces and the pulmonary capillary bed compared with full-term newborns impair lung function after preterm birth and make the immature lung vulnerable to mechanical damage (Langston et al 1984, Hansen and

Corbet 1998). In addition to surfactant deficiency, the immature lung lacks adequate antioxidant defence systems and there is imbalance of the protease-antiprotease system, and these render the lung more vulnerable to the toxic effects of oxygen (Hansen and Corbet 1998).

A preterm infant with surfactant deficiency may develop respiratory distress syndrome (RDS). The primary reason for the RDS is surfactant deficiency, caused by lower absolute amounts or by abnormal composition and function of the surfactant. The incidence of RDS is 60% at 29 weeks´gestation but declines with maturation to near 0 by 39 weeks. On histological examination, the peripheral air spaces are collapsed, respiratory bronchioles lined with necrotic epithelium and hyaline membranes have an overdistended appearance. There is obvious pulmonary oedema with congested capillaries, amd the lymphatic and interstitial spaces are distended with fluid. Reduced lung compliance ($C_L$), reduced functional residual capacity of the lung (FRC) and disturbed gas diffusion, but no increase in airway resistance ($R_{aw}$), are the functional features of the RDS (Hansen and Corbet 1998).

RDS is by far the most common cause of acute lung injury and respiratory failure in the small preterm infants (birth weight $\leq$ 1500 g). However, extreme immaturity itself, patent ductus arteriosus, apnoeic spells, aspiration, pulmonary infections, and condition worsening the general condition of neonate (e.g. severe asphyxia, intracranial haemorrhage, necrotizing enterocolitis, septicaemia) can also lead to respiratory insufficiency necessitating ventilatory support (Hansen and Corbet 1998). The degree of acute lung injury superimposed at a stage of incomplete lung development seems to play an important role in determining the severity of acute respiratory distress and whether bronchopulmonary dysplasia (BPD) or chronic lung disease (CLD) will develop.

### 2.1.2. Bronchopulmonary dysplasia and chronic lung disease of the newborn

As first characterized by Northway (1967) in a report on 32 infants, BPD is a chronic cardiopulmonary disease of infancy that follows ventilator and oxygen therapy for neonatal respiratory distress. In his initial report, Northway (1967) proposed that oxygen toxicity plays

a major role in the pathogenesis of BPD. Some other investigators, however, emphasized the role of intermittent positive pressure ventilation as the cause of BPD (Taghizadeh and Reynolds 1976). Since this initial description of Northway (1967), much has been learned about the pathogenesis, pathophysiology and outcome of BPD and it has been realized that BPD may also occur in infants who have not previously had RDS (Bancalari et al 1979, Koops et al 1984, O´Brodovich and Mellins 1985, Merritt et al 1988, Greenough 1990a, Holzman and Frank 1992, Northway 1992, Rojas et al 1995).

For purposes of uniform reporting, the initial definition of BPD of Bancalari (1979) was changed to a definition of disturbed gas exchange in an infant requiring oxygen at more than 28 days after birth (Sinkin 1990). However, this definition has become less useful because of the survival of infants at ever-lower gestational ages. This definition includes a wide range of infants, from those who ultimately appear to have no residual problems at one extreme to those with severe BPD, as described by Northway (1967). A more practical definition would be respiratory sequelae in an infant who is dependent on supplementary oxygen at 36 weeks´ postconceptional age (Shennan et al 1988). Using this definition showed that extending the time period to 36 weeks´ postconceptional age improves the predictive power of supplementary oxygen dependence (Shennan et al 1988). A recent study indicates that radiographic evidence consistent with BPD was more predictive of long-term respiratory outcome than other commonly used criteria (Palta 1998). However, differences in diagnostic criteria of BPD between hospitals and physicians cannot easily be eliminated, and population-based studies of neonatal outcome and studies of incidence are especially difficult to compare.

The incidence of BPD is generally reported at 20% of ventilated newborns, but wide variability exists between centers because of differences between populations and managements (Harrod et al 1974, Bancalari et al 1979, Avery et al 1987, Sinkin et al 1990, Abman and Groothius 1994). The incidence is inversely related to birth weight, being as high as 85% of neonates between 500-699 g but decreasing to 5% in infants with birthweights over 1500 g (Avery et al 1987, Parker et al 1992). With the survival of increasing numbers of infants of 24 to 26 weeks´ gestation, the numbers of infants with significant BPD have also

risen markedly (Zimmerman and Farrell 1994).

*Pathological stages:* Northway et al (1967) divided the clinical, radiological, and pathological progression of BPD into four stages. Stage 1 (at 1 to 3 days of age) consisted of the initial picture of RDS and revealed marked alveolar and interstitial oedema with hyaline membranes, atelectasis and necrosis of the bronchial mucosa. During stage 2 (at 4 to 10 days of age), atelectasis becomes more extensive, alternating with areas of emphysema. There is widespread necrosis and repair of bronchial mucosa. Cellular febris fills the airways. There is increasing opacification of the lungs. Stage 3 (at 11 to 30 days) is a transition period to chronic lung disease with radiological changes characteristic of interstitial fibrosis, emphysematic areas and atelectasis foci. Extensive broncial metaplasia and hyperplasia evolve. This stage could be complicated by patent ductus arteriosus (PDA) and congestive heart failure. During stage 4 (after 30 days of age) there is massive fibrosis of the lung with destruction of alveoli and airways. There is hypertrophy of bronchial smooth muscle, metaplasia of airway mucosa, and actual loss of pulmonary arterioles and capillaries and medial muscular hypertrophy of remaining vessels. The electrocardiogram at this stage usually shows right ventricular hypertrophy and cardiomegaly with cor pulmonale (Northway et al 1967). Today, the term chronic lung disease of prematurity is used widely instead of BPD (Hudak et al 1992). Sometimes the term BPD is used to represent only the more severe end (Northway stage IV) of the spectrum of chronic lung disease in infants born preterm (Greenough 1990a). The term CLD, used to describe the aftermath of preterm birth and its treatment, is deliberately vague (Kotecha and Silverman 1999). CLD is currently a widely used definition to embrace both a range of well-characterized syndrome and an overall increase in respiratory tract morbidity in infancy and later life.

*Etiology and pathogenesis*: Premature birth is associated with immature lung structure, lack of surfactant, and inadequate respiratory drive, all of which contribute to respiratory failure. In the multifactorial pathogenesis of CLD, respiratory failure is critical because it requires treatment with supplementary oxygen and artificial ventilation. CLD represents the developing lung´s response to acute lung injury and altered repair processes. The principal factors in the pathogenesis of CLD are genetic predisposition, immaturity of the lung, which

contributes to chronic pulmonary insufficiency of prematurity, oxygen toxicity, barotrauma, inflammation, pulmonary oedema and PDA and perhaps infection (Merrit et al 1988, O´Brodovich and Mellins 1985, Abman and Groothius 1994, Watterberg et al 1996, Hansen and Corbet 1998). These factors induce typical histological features of CLD. Marked airway changes, such as squamous metaplasia of large and small airways, increased peribronchial smooth muscle with fibrosis, chronic inflammation, and submucosal oedema with hypertrophied submucosal glands. Parenchymal disease is characterized by volume loss from atelectasis and alveolar septal fibrosis alternating with regions of hyperinflation and emphysema. The number of alveoli is markedly diminished, the internal surface area of the lung is decreased and lung volume is diminished (Bonikos et al 1976, Sobonya et al 1982, Stocker 1986, Marcgraft et al 1991). This is associated with decreased numbers of small pulmonary arteries. Structural changes in the pulmonary circulation include intimal proliferation, smooth muscle hypertrophy and advential thickening (Tomashefski et al 1984, Hislop and Haworth 1990).

Although, many aspects of CLD following neonatal intensive care have changed since Northway´s classic description, high morbidity and mortality persist, although there are changing trends in the epidemiology and pathogenesis of neonatal CLD. New therapies, such as administration of exogenous surfactant (Pramanik et al 1993), high frequency ventilation (HiFi study group 1990), pre- and postnatal steroids and other changes in patient care may have altered the severity of CLD, but CLD still remains a major clinical problem. With continued improvement in the survival of extremely preterm newborns, CLD remains one of the most significant sequelae of neonatal intensive care. One recent study suggested that much of the increase in the incidence of CLD may be because of improved survival (Parker et al 1992). In recent years, less severe forms of CLD have frequently been observed in infants with mild or no initial RDS (Heneghan et al 1986, Parker et al 1992, Rojas et al 1995). This change in clinical pattern suggests that, in these infants, risk factors different from those associated with the severity of the initial respiratory course are responsible for the development of CLD (Merth et al 1997). It also suggests a different aetiology for CLD in the smallest babies (<700 g), who have a high risk of CLD without antecedent acute pulmonary disease and need prolonged mechanical ventilation (Kotecha and Silverman 1999)

### 2.1.3. Abnormalities of lung function during infancy in children born preterm

The most prominent lung function abnormalities in preterm born infants with CLD are increased $R_{aw}$ and decreased specific airway conductance ($SG_{aw}$) (Ahlström 1975, Stocks et al 1978, Morray et al 1982, Goldman et al 1983, Lindroth and Mortensson 1986, Gerdhard et al 1987, Motoyama et al 1987, Hifi Study 1990, Lebourges et al 1990, Yuksel et al 1991, Van Lierde et al 1994, Baraldi et al 1997) and bronchial hyperreactivity (BHR) (Motoyama et al 1987). Initially low $SG_{aw}$ reaches the range of normality by the age of 2-3 years (Bryan et al 1973, Gerdhard et al 1987, Baraldi et al 1997). As defined by responsiveness to bronchoconstrictor (methacoline, histamine, cold air) challenges, BHR persists in older children and adolescents with a history of CLD (Smyth et al 1981, Morray et al 1982, Bader et al 1987, Andreasson et al 1989, Kleine et al 1990, Northway et al 1990, Blayney et al 1991, Mallory et al 1991). Pulmonary function measurements have also characterized other abnormalities of lung mechanics, including increased dead space ventilation, decreased $C_L$, maldistribution of ventilation, increased work of breathing and abnormal ventilation-perfusion matching (Hansen and Corbet 1998). Serial studies during infancy and early childhood demonstrate that lung volumes (FRC) are decreased in early infancy but increase with time (Bryan et al 1973, Gerdhard et al 1987, Baraldi et al 1997) probably by formation of new alveoli. Some investigators have not found increased FRC during late infancy (Tepper et al 1986). Total respiratory system compliance is lower in CLD infants than in controls, probably reflecting mechanical alteration in the lung´s elastic properties (Stocker 1986) and loss of lung tissue (Van Lierde et al 1994), but has a tendency to normalize with advancing age (Bryan et al 1973, Ahlström 1975, Stocks and Godfrey 1976, Baraldi et al 1997). Preterm infants with RDS but without CLD may also have abnormal lung function (Wong 1982, Yuksel and Greenough 1992a, Merth et al 1997).

However, although pulmonary mechanics in CLD survivors tends to improve during the early years of life, reaching the range of normal values, 70% of these children still show abnormal lung function when this is evaluated from forced expiratory flow by the squeeze technique at 2 years of age (Tepper et al 1986, Baraldi et al 1997). These findings support the prediction that early injury to the airways tends to persist in infancy (Motoyama et al 1987) and

represents a risk factor for obstructive airway disease later in childhood and adolescence (Northway et al 1990).

## 2.2. Abnormalities of lung function in schoolchildren born preterm

Although many abnormalities of pulmonary function in preterm born infants can improve with age (Gerdhard et al 1987, Mallory et al 1991, Blayney et al 991, Koumbourlis et al 1996, Baraldi et al 1997) there is increasing evidence that respiratory problems persist into late childhood and early adolescence.

### 2.2.1. Basic lung function

Abnormal spirometric values, indicating bronchial obstruction, have been reported by several investigators in schoolchildren born preterm with a history of CLD (Smyth et al 1981, Bader et al 1987, Andreasson et al 1989, Northway et al 1990, Kleine et al 1990, Hakulinen et al 1990, Blayney et al 1991, Parat et al 1995, Hakulinen et al 1996, Koumbourlis et al 1996, Jacob et al 1997, Jacob et al 1998). The largest study so far is a group of preterm children with CLD monitored by Gross et al (1998). At 7 years of age the 43 children with a history of CLD had more airway obstruction, i.e. significantly reduced forced vital capacity (FVC), forced expiratory volume in one second ($FEV_1$) and forced expiratory flow at 50-75% of vital capacity ($FEF_{25-75}$) values, than either 53 preterm children without a history of CLD or 108 children born at term. Increased residual volume of the lung and total lung capacity ratios (RV/TLC) or elevated FRC, indicating air-trapping, is a common finding in children with a history of CLD (Smyth et al 1981, Bader et al 1987, Andreasson et al 1989, Northway et al 1990, Hakulinen et al 1990, Blayney et al 1991, Koumbourlis et al 1996, Jacob et al 1998). Those children with the greatest degree of airflow limitation and air-trapping had the greatest abnormalities in both the shape and position of the pressure volume curves of the lung (Jacob et al 1998).

The long-term pulmonary outcome in preterm children with a history of RDS but without a

history of CLD is less clear. In earlier studies, children with histories of RDS had normal pulmonary function by school age (Lamarre et al 1973, Stahlman et al 1982). In studies in which children with more immature lung have been followed, reduced flow and $SG_{aw}$ values and elevated RV/TLC ratios have been found in ventilator-treated children at school age compared to controls born at term (Bertrand et al 1985, Galdes-Sebaldt et al 1989, Chan et al 1989c, Lebourges et al 1990, Kleine et al 1990, Hakulinen et al 1996, Jacob et al 1997). Cano and Payo (1997) found that children born preterm with RDS but without CLD at the age of 11 years had significantly reduced flow values and a significantly higher $R_{aw}$ compared with those children without RDS, not ventilated for other causes, and matched for gestational age. In contrast, among preterm born schoolchildren without previous CLD, but RDS in 38% of these children, Gross et al (1998) found that lung function was similar to that of the term control group.

Prematurity alone is also assumed to be a risk factor for abnormal lung function in childhood. Studies of preterm born schoolchildren without significant respiratory disease have shown reduced $FEV_1$, $FEF_{50-25}$ , $SG_{aw}$ values, and abnormal lung mechanics (Coates et al 1977, Bertrand et al 1985, Mansell et al 1987, Galdes-Sebaldt et al 1989, Parat et al 1995). Galdes-Sebaldt et al (1989) and Bertrand et al (1985) found increased RV/TLC ratio in those children. Chan et al (1989c) reported that their preterm born children had reduced $FEV_1$ values at 7 years of age, irrespective of whether they had required ventilation in the neonatal period. In contrast, Northway et al (1990) found that the pulmonary function of their preterm born control children who had not undergone mechanical ventilation had lung function similar to that of controls born at term. In a recent large, population-based Scottish study of 300 very low birth weight (VLBW) children of 8 to 9 years of age, FVC was more affected than expiratory function (McLeod et al 1996). In contrast, Kitchen et al (1992) reported that in their VLBW population there was little evidence of impared lung function at 8 years.

The effect of intrauterine growth on lung function at school age is controversial. In a large study of schoolchildren throughout the United Kingdom, Rona et al (1993) reported that birth weight for gestational age, i.e. intrauterine growth, was significantly associated with FVC and $FEV_1$ after taking gestational age, parental smoking, and social factors into account. In

contrast, McLeod et al (1996) concluded that poorer lung function of their VLBW population is associated with very low birth weight, but not with intrauterine growth retardation. In their population, 34% of children were small for gestational age but did not have reduced lung function as compared with appropriately grown VLBW children. Thus, they could not confirm the association of obstructive airway disease with intrauterine growth retardation reported in some studies (Barker et al 1991, Rona et al 1993, Nikolajev et al 1998). Matthes et al (1995) also reported that low birth weight at term was not associated with reduced lung function in adolescents.

### 2.2.2. Response to $\beta_2$ agonist and to the bronchial provocation tests

Although many schoolchildren born preterm may have airway obstruction regardless of CLD, there may be a reactive component, as indicated by the bronchodilator response in some subjects. In a recent study, 57% of all children, not only those with a history CLD but also preterm children without a history of CLD in the control group, showed a significant response to bronchodilation (Jacob et al 1998). Gross et al (1998) observed bronchodilator responsiveness twice as often in preterm children with previous CLD (47%) as in preterm children without a history of CLD (25%). However, they did not test bronchodilator responsiveness in all children irrespective of spirometry results. In earlier studies at least half the preterm born schoolchildren with a history of CLD had significant bronchodilator responsiveness (Smyth et al 1981, Andreasson et al 1989, Northway et al 1990, Kleine et al 1990, Koumbourlis et al 1996). In addition, some children born preterm without a history of CLD may have had a significant bronchodilator response (Andreasson et al 1989).

Increased incidence of BHR, as demonstrated by positive methacoline or histamine challenges, has been observed in children born preterm, irrespective of their RDS or CLD status (Bertrand et al 1985, MacLusky et al 1986, Riedel 1987, Chan et al 1988, Chan et al 1989a, Galdes-Sebaldt et al 1989). Cano and Payo (1997) found BHR in 35% of preterm born children with previous RDS but without a history of CLD and in 23% in children without RDS, but they did not have a control group born at term. In studies of a group of children with a history of CLD, the majority of children had BHR (Smyth et al 1981, Bader et al 1987,

Blayney et al 1991, Koumbourlis et al 1996). Northway et al (1990) also found that adults with a history of CLD had BHR, but their preterm control group did not differ from their control group born at term. Although lung function abnormalities in children with a history of CLD may improve with age, BHR persists (Blayney et al 1991). Duiverman et al (1988) found normal bronchial responsiveness in 3- to 10-year-old preterm born children tested by the forced oscillation technique.

### 2.2.3. Diffusing capacity

Smyth et al (1981) measured the pulmonary diffusing capacity of carbon monoxide ($D_{LCO}$) in five children with a history of CLD aged 7-9 years and found normal results. Galdes-Sebaldt et al (1989) found significantly decreased $D_{LCO}$ values in 11- to 13-year-old children with birth weights of < 1500 g who had suffered from neither RDS nor CLD. Northway et al (1990) found slightly lowered $D_{LCO}$ values in young adults with a history of CLD. In a Finnish study, $D_{LCO}$ values in preterm born children both with and without a history of CLD were significantly lower than those in controls born at term (Hakulinen et al 1996). However, the diffusing capacity per effective alveolar volume ($D_L/V_A$), did not differ significantly between the groups (Hakulinen et al 1996). In a recent study, no significant difference in $D_{LCO}$, either uncorrected or corrected for lung volumes, was found in subjects with a history of CLD as compared with a preterm born group without a history of CLD (Jacob et al 1998). However, these values were not compared with a term control group.

### 2.2.4. Testing during exercise

In several studies it has been observed that even children with a history of severe CLD did not differ from either preterm or term-born control children in their maximal work capacity or maximal oxygen consumption (Bader et al 1987, Andreasson et al 1989, Parat et al 1995, Jacob et al 1997, Gross et al 1998). However, children with CLD used a greater percentage of their ventilatory reserve. These children were also more likely than control children to have oxygen desaturation during exercise, and this was most evident in children with markedly reduced values for $FEV_1$ (Jacob et al 1997). Bader et al (1987) found that exercise-induced

bronchospasm occurred in 50% of the CLD group but not in the control group born at term indicating BHR.

Some investigators have found a significant correlation between current measures of reduced airflow and the duration of mechanical ventilation or the time on supplementary oxygen (Stahlman et al 1982, Arad et al 1987, Bader et al 1987, Andreasson et al 1989, Jacob et al 1998). Some have failed to find any such association (MacLusky et al 1986, Chan et al 1989c, Galdes-Sebaldt et al 1989, Hakulinen et al 1996, Cano and Payo 1997). Chan et al (1989c) found a strong correlation between birth weight and $FEV_{0.75}$ at 7 years of age in agreement with several other studies (Bader et al 1987, Mansell et al 1987, Galdes-Sebaldt et al 1989). Male sex was also independently associated with reduced $FEV_{0.75}$ (Chan et al 1989c). Birth weight was observed to be associated with $D_{LCO}$ values at school age (Galdes-Sabaldt et al 1989, Hakulinen et al 1996)

## 2.3. The role of inflammation in the pathogenesis of abnormal lung function

### 2.3.1. In children born preterm

There is growing evidence suggesting that an inflammatory pulmonary reaction following lung trauma may be an early event in the course of CLD, finally leading to lung fibrosis (Groneck and Speer 1995). Inflammation includes production of chemotactic factors in response to a specific stimulus (trauma, hypoxia/reperfusion, hyperoxia), migration of inflammatory cells, increased expression of adhesion molecules on endothelial and epithelial cells, adhesion of neutrophils, secretion of proteases and toxic oxygen radicals and increased microvascular permeability (Groneck and Speer 1995). In response to a primary form of injury, oxygen toxicity or volutrauma, there is an inflammatory response (Merritt et al 1981, Odgen et al 1984, Pierce and Bancalari 1995) which is reflected in increased numbers of neutrophils in the bronchial lavage samples as early as the second day of life (Odgen et al 1984, Arnon et al 1993). In the bronchial lavage sample, the neutrophil count peaks on the fourth day of life and then declines rapidly to normal by the end of the first week in those who

recover from RDS but declines much more slowly in those who go on to develop CLD (Odgen et al 1984). In parallel to the neutrophil influx, the chemotactic activity of tracheobronchial aspirate fluid is increased in infants developing CLD (Groneck et al 1994). In the bronchial lavage samples during the first week, there are elevated levels of mediators and cytokines, such as leukotrienes, platelet-activating factor, fibronectin, fibroblast-activating factors and others (Groneck and Speer 1995, Hansen and Corbet 1998); elevated levels of interleukins, especially interleukin-8 (Groneck et al 1994, Kotecha et al 1995, Munshi et al 1997); and in one of the earliest responses to injury, markedly elevated levels of interleukin-6 in bronchial lavage samples on the first day of life in those destined to develop CLD (Bagchi et al 1994). Recruitment of neutrophils to the lung is thought to occur via interactions occurring between adhesion molecules on the surfaces of endothelial cells and neutrophils; increased concentrations of the adhesion molecules have been found in the tracheal aspirates of infants with early CLD (Kotecha et al 1995).

High levels of neutrophil elastase and desmosine (a degradation product of elastin) were found in airway specimens of infants with CLD (Merritt et al 1983, Bruce et al 1985, Speer et al 1993, Groneck and Speer 1995) and the activity of an important antiprotease was low (Odgen et al 1984). An imbalance between protease and antiprotease activity was therefore suggested to be an important mechanism of lung damage in CLD. The ratio of elastase to secretory leucocyte protease inhibitor was higher in infants who developed CLD than in controls, suggesting an unfavourable balance (Merritt et al 1983, Watterberg et al 1994a). At present there is only an association between pulmonary inflammation and microvascular permeability, one of the most important pathophysiological factors of early CLD; clear causality has not proved (Groneck and Speer 1995). In infants who do not develop CLD, the neutrophils undergo programmed cell death (apoptosis) and are ingested by macrophages without releasing their elastases or other destructive enzymes (Grigg et al 1991); this process may be disturbed in those who develop CLD. Studies have also reported not only persistent elevation of neutrophils but also activated alveolar macrophages in the tracheal fluid effluent of infants with CLD (Groneck and Speer 1995). In children with CLD in whom bronchoalveolar lavage was performed at a mean age of 4 months, alveolar macrophages represented 90% of the cell population (Clement et al 1988). Activated macrophages can

produce oxygen free radicals and excess fibronectin, which is a potent chemoattractant and growth factor for fibroblasts. Overstimulation of the repair response with increased production of cellular fibronectin is associated with the development of pulmonary fibrosis, which is prominent in infants who die of CLD (Pierce and Bancalari 1995). In recent studies it has been suggested that, in CLD, eosinophils also participate in the inflammatory process (Yamamoto et al 1996, Raghavender and Smith 1997).

It is assumed that these early inflammatory changes in ventilator-dependent neonates and subacute inflammatory changes in wheezy preterm infants are important pathological features contributing to the abnormal lung function findings. In contrast, it is suggested that abnormalities of pulmonary function and increased BHR in preterm born schoolchildren are not solely an effect of chronic bronchial inflammation but are due to abnormal changes in pulmonary structure (Chan and Silverman 1993). Although the abnormalities of pulmonary function are similar to those seen in asthma, on the ground of the response to GC treatment it is suggested that chronic bronchial inflammation does not play a part in these changes (Chan and Silverman 1993).

### 2.3.2. In adults with asthma or with chronic obstructive pulmonary disease

Eosinophil-mediated damage to the respiratory epithelium is a major pathogenetic mechanism in asthma (Bousquet et al 1990). Activated eosinophils secrete several granule-derived proteins, e.g. eosinophil cationic protein (ECP) (Venge 1994), which is increased in asthma (Bousquet et al 1990, Lacoste et al 1993). In chronic obstructive pulmonary disease (COPD) of adults, neutrophils are prominent in the airways (Lacoste et al 1993). Activated neutrophils secrete several proteins including myeloperoxidase (MPO) (Venge 1994), which is increased in bronchoalveolar lavage fluid in COPD (Lacoste et al 1993).
Numerous studies have shown that lymphocytes also play an important role in the pathogenesis of asthma (Azzawi et al 1990, Robinson et al 1991) and also in COPD (O´Shaughnessy et al 1997, De Jong et al 1997). In adults with mild to severe asthma, both activated T cells (activated $CD4^+$ T lymphocytes, as reflected by an increase in the expression of CD25) and activated eosinophils have been observed in bronchial biopsies (Bousquet et al

1990, Azzawi et al 1990) in bronchoalveolar lavage (BAL), and in the peripheral blood of adult patients (Bousquet et al 1990, Robinson et al 1991, Wilson et al 1994). The degrees of T-lymphocyte activation and eosinophilia were closely correlated with the severity of the asthma and the degree of bronchial responsiveness (Bousquet et al 1990, Azzawi et al 1990, Robinson et al 1991). Further, the percentages of activated T cells decreased with inhaled GC treatment (Wilson et al 1994). These findings suggest that T-cell activation and eosinophil recruitment represent a pathway leading to the development of airway narrowing, hyperresponsiveness, and symptoms of atopic asthma (Bousquet et al 1990, Azzawi et al 1990, Robinson et al 1991, Wilson et al 1994).

However, there have been few studies on the pathogenesis of childhood asthma. The properties of peripheral blood cells in asthmatics closely resemble those of cells in the bronchial mucosa and BAL fluid, suggesting that peripheral blood could be used for immunological studies (Gemou-Engesaeth et al 1997). Childhood asthma, like asthma in adults, is associated with activation of T cells and elevated expression of eosinophil-active cytokines. Furthermore, GC therapy results in clinical improvement and in a decrease in T-cell activation (Gemou-Engesaeth et al 1997). The few studies on the role of lymphocytes in smoking-induced airflow limitation in COPD have shown a tissue predominance of $CD8^+$ over $CD4^+$ T cells (O´Shaughnessy et al 1997), and a parallel increase in $CD8^+$ lymphocytes in peripheral blood (De Jong et al 1997). Furthermore, the presence of bronchial wall macrophages is evident (O´Shaughnessy et al 1997). These data support the view that the pattern of inflammatory cells in COPD is different from that in asthma. In COPD, there is also eosinophil migration towards the airways, but the phenomenon is more pronounced in asthma (Lacoste et al 1993). There have been no studies on the role of lymphocytes in the pathogenesis of lung function abnormalities in children born very preterm.

## 2.4. Exogenous surfactant therapy in respiratory distress syndrome

### 2.4.1. Surfactant trials

Surfactant substitution has become an established treatment for neonatal RDS (Soll et al

1992). Since the pioneering efforts of Robillard et al (1964), Chu et al (1967) and Fujiwara et al (1980), numerous randomized, controlled trials have demonstrated the immediate effectiveness of surfactant therapy in the treatment of infants with established RDS (Soll et al 1992). The therapeutic approach has followed two general strategies: administration of exogenous surfactant within 30 minutes of birth to small preterm infants at high risk of RDS ("prophylactic") and administration of surfactant a few hours after birth to neonates with RDS in respiratory failure ("rescue"). In randomized clinical trials, synthetic, natural and modified natural surfactants were used. Surfactant administration at birth or in established respiratory failure decreases the severity of RDS, the frequency of air leaks (pneumothorax and pulmonary interstitial emphysema) and neonatal mortality (Soll et al 1992, Jobe et al 1993, Horbar et al 1993, Schwartz et al 1994). These benefits outweigh the small increase in pulmonary haemorrhage associated with surfactant supplementation (Raju and Langenberg 1993). In spite of the use of surfactant preparations, CLD continues to develop in a significant number of preterm infants. Many observers now accept that surfactant does not reduce the overall incidence of CLD, in part because increases in the overall survival rate create a larger population at risk of long-term complications (Hudak et al 1992, Jobe et al 1993). However, the incidence of survival without CLD is significantly increased (Pramanik et al 1993), and in rescue trials with synthetic surfactant, the absolute incidence of CLD appeared to be significantly reduced (Corbet et al 1993, Jobe et al 1993). According to many studies, surfactant given at birth or very early in respiratory failure is associated with a better outcome than surfactant given later after birth (Kendig et al 1991, The OSIRIS collaborative Group 1992, Kattwinkel et al 1993, Halliday 1995, Cooke 1995, Egberts et al 1997).

### 2.4.2. Effect on pulmonary outcome

Administration of surfactant to preterm infants with RDS increases the surface activity at the air-liquid interface, causing an immediate and dramatic improvement in oxygenation and in most cases reducing the need for ventilatory support (Soll et al 1992). This can be directly attributed to stabilization of alveoli and decreased elastic recoil, allowing for better maintenance of functional residual capacity (Goldsmith et al 1991). Surfactant therapy improves respiratory compliance and lessens the effort of breathing during the first week of

life (Couser et al 1990, Morley and Greenough 1991, Bhutani et al 1992). After surfactant supplementation, the dynamic respiratory compliance of ventilated preterm infants either improved after a delay of up to 24 hours (Couser et al 1990, Morley and Greenough 1991, Bhutani et al 1992, Kelly et al 1993) or showed no detectable change (Davis et al 1988). These modest changes in dynamic compliance may be due in part to distension of the lung and an increase in FRC following surfactant therapy (Milner 1993). The improvement in pulmonary function may be short-lived (Davis et al 1988, Couser et al 1990, Morley and Greenough 1991, Bhutani et al 1992, Goldman et al 1992) and no longer detectable at 28 days of age (Goldman et al 1992). The improved pulmonary outcome of the surfactant recipients may have been due to the immediate improvement in lung function, resulting in shorter exposure to high levels of inspired oxygen and to high ventilatory pressures. Additionally, surfactant may protect against development of epithelial lesions of the airways during artificial ventilation (Robertson 1992) and may ameliorate hyperoxic lung injury (Fracica et al 1994). Both barotrauma and hyperoxia cause significant injury to the lungs and airways (Jackson et al 1990).

The long-term effects of surfactant therapy on lung function vary, perhaps because of differences in the techniques used for measuring lung function, differences in the patients studied or differences in treatment practice, including type and dosage of surfactant. Yuksel et al (1993) and Abbasi et al (1993) found evidence that synthetic surfactant improved lung function compared with a placebo group at follow-up after 7-12 months of age. Abbasi et al (1993) found that infants treated with Exosurf had lower $R_{aw}$ and higher forced expiratory flow, indicating that there was less injury to the conducting airways with surfactant treatment. In contrast, the results of Walti et al (1992) and Couser et al (1993) suggest that exogenous porcine (Walti et al 1992) or bovine (Couser et al 1993) surfactant therapy had no effect on pulmonary function at 1 to 2.5 years of age in infants surviving RDS. Preliminary data with controversial results are also available on the long-term pulmonary outcome of surfactant treatment in children at school age (Kramer et al 1995, Berner et al 1996).

## 2.5. Postnatal glucocorticoid therapy in children born preterm

### 2.5.1. Controlled trials with dexamethasone

The incidence of CLD in infants born very preterm remains distressingly high in spite of the reduction in acute morbidity and mortality resulting from the recent introduction of surfactant treatment for RDS (Soll al 1992). A number of clinical trials have shown that dexamethasone is effective in shortening the time of extubation and the length of time the patient remained on mechanical ventilation in ameliorating established CLD (Mammel et al 1983, Avery et al 1985, Harkavy et al 1989, Cummings et al 1989, Kazzi et al 1990, The Collaborative Dexamethasone Trial Group 1991, Kari et al 1993, Kothadia et al 1999). Dexamethasone also improves lung mechanics, increasing static $C_L$ (Yoder et al 1991, Brundage et al 1992) and reducing $R_{aw}$ (Brundage et al 1992). The mechanism involved in the improvement of pulmonary function in CLD is currently poorly understood. The mechanics of action of dexamethasone might be suppression of lung inflammation (Yoder et al 1991, Watts et al 1992). Others have suggested suppression of lung collagen synthesis (Co et al 1993) or reduced protein leakage resulting in decreased surfactant inhibition (Kari et al 1994). Low plasma cortisol values have been documented during the first few days of life in VLBW infants who subsequently experienced CLD (Watterberg al 1994b) indicating GC therapy. In contrast, Ballard et al (1996) found no difference in cortisol levels between infants who went on to develop CLD and those who did not.

Cellular and biochemical studies have shown inflammatory changes in the lungs of ventilated infants as early as the first few days of life (Merritt et al 1981, Odgen et al 1984, Pierce et al 1995) and dexamethasone has been shown to reduce this inflammation (Yoder et al 1991, Gronec et al 1993). Thus it has been postulated that steroid treatment administered shortly after birth may prevent the inflammatory insult to the lung and thereby reduce the likelihood of CLD (Yeh et al 1990). Previous reports of the use of early postnatal dexamethasone treatment have provided mixed results. There is evidence to support its role in reducing the incidence of CLD when treatment is begun during the first week of life (Yeh et al 1990, Brozanski et al 1995, Durand et al 1995, Rastogi et al 1996, Yeh et al 1997, Lin et al 1999,

Garland et al 1999). Some recent studies have shown that dexamethasone given to preterm infants with RDS did not reduce the incidence of CLD and/or death (Sanders et al 1994, Shinwell et al 1996, Subhedar et al 1997, Tapia et al 1998). Comparisons of the results of controlled trials on postnatal dexamethasone given during the first few days of life to preterm infants with RDS are difficult to make because of substantial differences in study design. The duration and the time of starting treatment, study population selection, simultaneous surfactant therapy and additional steroid therapy may all affect the result. In addition, the definition of CLD differs between studies. However, a recent meta-analysis of systemic GC therapy within the first 2 weeks of life in infants born preterm concluded that this therapy significantly reduced the risk of CLD. When started between 7 and 14 days of age, there was also a significant reduction in mortality (Bhuta and Ohlsson 1998).

The short-term side effects of dexamethasone include hyperglycaemia (Harkavy et al 1989, Kazzi et al 1990, Collaborative Dexamethasone Trial Group 1991, Bhuta and Ohlsson 1998, Papile et al 1998), hypertension (Avery et al 1985, Cummings et al 1989, Collaborative Dexamethasone Trial Group 1991, Bloomfield et al 1998, Bhuta and Ohlsson 1998, Papile et al 1998), neutrophilia and infection (Yeh et al 1997, Papile et al 1998), diminished weight gain (Harkavy et al 1989, Yeh et al 1990, Gibson et al 1993, Shrivastava et al 1998, Bhuta and Ohlsson 1998, Papile et al 1998), reduced bone mineral content (Shrivastava et al 1998), myocardial hypertrophy (Bensky et al 1996, Bloomfield et al 1998), gastrointestinal bleeding/perforation (Ng et al 1991), adrenal suppression (Wilson et al 1988, Bloomfield et al 1998) and diminished head growth (Papile et al 1998). Despite the wide use of postnatal dexamethasone in the treatment of CLD, the long-term effects of dexamethasone therapy have not been well studied, although concern has been emphasized by various investigators (Kazzi et al 1990, Frank 1991, Jones et al 1995, Yeh et al 1997). In a recent study in preterm born infants a 28-day course of systemic dexamethasone started on day 1 of life has been associated with a higher incidence of neuromotor dysfunction at 2 years of age (Yeh et al 1998). O´Shea et al 1999 found a higher rate of cerebral palsy among dexamethasone-treated infants at 1 year of age. In an animal model disturbances in lung growth, myelinization and neurodevelopment have been observed (Howard et al 1975, Weichsel 1977, Beck et al 1981). Pulse therapy of dexamethasone in preterm infants at risk for CLD has fewer side effects than

a long course but may be less effective in preventing CLD (Brozanski et al 1995, Bloomfield et al 1998).

### 2.5.2. Trials with inhaled glucocorticoid

Recently, inhaled GC has been proposed as an alternative treatment for infants born preterm to minimize the systemic complications that occur with parenteral GC. There have been few controlled trials of inhaled GC in the treatment of newborns with lung disease. Improvements were found in oxygen requirements, $R_{aw}$ and dynamic $C_L$ and time to extubation in comparison with controls without GC side effects (Pappagallo et al 1991, LaForce and Brudno 1993, Pokriefka et al 1993, Arnon et al 1996, Fok et al 1999). In a recent study, 253 infants born before 33 weeks requiring ventilation therapy at 3 to 14 days of age were treated with inhaled beclomethasone or placebo for 4 weeks. In this study, early beclomethasone therapy did not prevent CLD but was associated with lower rates of use of systemic GC therapy and mechanical ventilation (Cole et al 1999). Studies of inhaled GC in infants born preterm have shown promising results with minimal side effects. However, there are limitations associated with the route of administration and a delayed effect. With systemic steroids, extubation can be performed as early as 48 hours; in contrast, with inhaled steroid, the improvement in pulmonary function is only visible after 2 weeks (LaForce and Brudno 1993). Early use of inhaled steroids does not prevent CLD (Cole et al 1999), which is achieved by the systemic route. The recent study by Groneck et al (1999) in which they compared the effects of 1500 μg/day of inhaled beclomethasone and systemic dexamethasone on clinical variables and lung inflammation support this finding (Gronec et al 1999). It is also important to determine the amount of aerolized drug delivered to intubated babies before clinical studies when using inhaled GC in the treatment of infants born preterm (Pelkonen et al 1997).

Very few data are available on inhaled GC treatment in older infants and children born preterm. Konig et al (1992) have described the use of nebulized flunisolide in older infants with CLD. In infants with steroid-dependent CLD between 7 and 18 months of age, the use of systemic GC has been reduced during inhalation therapy with beclomethasone dipropionate

(Cloutier et al 1993). Yuksel and Greenough (1992) demonstrated a reduction in symptom score and an increase in FRC produced by using beclomethasone dipropionate for 6 weeks in infants born preterm who continued to have respiratory symptoms at the age of 10.5 months despite bronchodilator therapy. However, Chan and Silverman (1993) selected 15 schoolchildren born preterm, aged 8.2 years, with respiratory symptoms and BHR for treatment with inhaled beclomethasone dipropionate in a dose of 0.4 mg daily or placebo in a crossover design for 4 weeks, and found no significant differences in respiratory symptoms, basic lung function or the response to histamine between these treatments. The authors suggested that the increased bronchial responsiveness in survivors of preterm birth at school age was not caused by bronchial inflammation, but was due to abnormal pulmonary development caused by preterm birth and neonatal intensive care. These result contrast markedly with the beneficial effects of corticosteroids on CLD in early infancy.

## 2.6. Asthma and children born preterm

*Family history of asthma and prematurity:* The relationship between prematurity and asthma is controversial. There are several studies on a maternal or a family history of asthma as a risk factor for prematurity, neonatal respiratory disease and CLD. Mothers of preterm born infants have been found to have a higher incidence of BHR than term infants and their mothers (Bertrand et al 1985, Riedel et al 1989, Kelly et al 1995). In contrast, Chan et al (1988) demonstrated that neither the prevalence of maternal asthma nor a family history of asthma was increased in infants born preterm. Thus whether there is an association between maternal BHR or asthma and premature birth remains controversial (Chan et al 1995). Studies that examined the relationship between a family history of asthma and neonatal respiratory disease in prematures were also inconclusive (Chan et al 1989a, von Mutius 1993, Hagan et al 1995). Giffin et al (1995) found that infants with a family history of atopy tended to be born at an earlier stage of gestation and had higher $R_{aw}$ at 1 year of age. Hagan et al (1995) suggested that prematurity itself is not associated with a family history of asthma, although in infants with CLD a family history of asthma may increase the risk of severe CLD. Nickerson and Taussig (1980) found that a history of physician-diagnosed asthma was more frequent among

relatives of infants with CLD than among those of infants without CLD. However, other subsequent studies have not confirmed their finding (Chan et al 1989a, de Winter et al 1995, Hagan et al 1995). In most studies, no increase in the incidence of atopy was found among preterm born schoolchildren (Riedel et al 1987, Chan et al 1989a, Northway et al 1990).

On the other hand, preterm birth is considered to be a risk factor for asthma (Seidman et al 1991). In their large population study, Seidman et al (1995) found that infants with birth weights of < 2500 g (n=1004) had a significantly increased risk of developing asthma by 17 years of age compared with the reference group with birth weights of 3000-3499 g (n=8369). Such an association had been suggested previously (Noble-Jamieson et al 1982).
*Asthma and CLD:* Most of the earlier studies extending to school age had shown that small groups of preterm born children with or without CLD had reduced lung function (see section 2). Bronchial obstruction, bronchial lability and BHR, the characteristic features of asthma (Ryan et al 1982), are common findings, regardless of CLD, although more pronouced in children with CLD. According to many studies, about half the preterm born schoolchildren with a history of CLD have a significant bronchodilator response (see section 2.2). In a recent study, Gross et al (1998) observed that half the children with a history of CLD had a bronchodilator response at 7 years. Additionally, they found that this bronchodilator response was not associated with a family history of asthma or with exposure to smoking. Lower respiratory tract symptoms such as cough and wheezing, i.e. asthma-like symptoms, are common in preterm born children, particularly in the early years of life (Ford et al 1986, Myers et al 1986, Lucas et al 1990, Greenough et al 1990b, Elder et al 1996). Although the prevalence of cough and wheezing seems to decrease after the first few years of life, some preterm born children have respiratory symptoms later in life, as well (Smyth et al 1981, Chan et al 1989b, Rona et al 1993). Elder et al (1996) found that the incidence of wheezing treated with bronchodilators was 14.5% in very preterm infants (n=560) and 3% in a cohort of term newborns (n=657) during the first year of life. Significant risk factors for recurrent wheezing were a parental history of asthma, siblings at home, and maternal smoking, with the addition of immaturity and neonatal lung injury. Low birth weight for gestation had no effect (Elder et al 1996). On the other hand,

it is known that a number of children who wheeze during infancy will develop asthma later in childhood (Martinez et al 1995).

Follow-up studies have shown that, in general, children born preterm have in later childhood a greater than expected incidence of BHR (Chan et al 1988), unrelated to neonatal respiratory events (Chan et al 1989a, Galdes-Sebaldt et al 1989, MacLusky et al 1986, Cano and Payo 1997), but related to prematurity (Chan et al 1988, Galdes-Sebaldt et al 1989). It has been suggested that airway inflammation in children born preterm is not a mechanism of BHR similar to asthma (Chan et al 1993). A significant relationship has been found between the presence of BHR and the duration of mechanical ventilation (Riedel et al 1987, Ahrens et al 1988). Increased prevalence of BHR was also found in young adults with a history of CLD (Northway et al 1990). A significant relationship between BHR and a personal history of wheezing has been found in schoolchildren born preterm (Chan et al 1989a). In children born preterm, BHR has been found to be significantly associated with reduced baseline lung function (Chan et al 1989a, Koumbourlis et al 1996).

## 2.7. Peak expiratory flow variability in asthma

The physiological hallmark of asthma is variable bronchial obstruction. All lung function studies of preterm born schoolchildren have been done only in the clinic. Monitoring pulmonary function throughout successive days in preterm born schoolchildren, as in asthma diagnosis and treatment, might provide information about bronchial lability that would probably missed with a single measurement obtained in the clinic. Peak flow meters have been available for this purpose. In adults, home recording of peak expiratory flow (PEF) is considered to be a useful aid in making the diagnosis, assessing the severity, and monitoring the treatment of asthma (Kerstjens et al 1994, Sears et al 1997). In children with asthma, diurnal variation in PEF correlates significantly with asthma symptoms and BHR (Gern et al 1994, Brand et al 1997). Although diurnal variation in PEF is considered useful in the diagnosis and treatment of asthma and also in large epidemiological studies in children (Timonen et al 1997), many basic questions concerning the validity of monitoring pulmonary function at home are still unanswered. Conventional recordings of PEF in asthma performed

with hand-held mini PEF meters, as compared with devices capable of electronic storage, may overestimate the number of recordings, containing a large number of retrospective and even invented data (Redline et al 1996, Verschelden et al 1996, Chowienczyk et al 1994, Quirce et al 1995). Compliance in performing spirometry may have decreased during long-term recordings. In both children and adults, studies comparing simple PEF meters and devices capable of electronic storage have shown that missing data increased significantly after 2 weeks of monitoring (Redline et al 1996, Verschelden et al 1996). Also, preliminary studies of the quality of home spirometry in children have shown a significant decrease in compliance after the first month of recording, without any significant change in technical ability to perform the spirometry (Wensley et al 1997). The breathing manoeuvre is strenuous and requires considerable effort, so co-operation may wane over time and submaximal efforts create falsely low PEF readings (Enright et al 1994).

For expiratory flow rates, normal circadian rhythms occur in the absence of external stimuli, with an afternoon maximum and an early morning minimum when plasma histamine is at its maximum and plasma cyclic adenosine monophosphate and epinephrine are at their minimum (Lebowitz 1991). This pattern is exaggerated in asthmatics and is a good indicator of bronchial lability. The presence of increased bronchial obstruction, reflected by reduced PEF in asthmatic patients during the early morning hours, is called the morning dip (Engricht et al 1994). The diurnal variation percentage or lability percentage is calculated for each day: the maximum PEF (usually noon or evening) is subtracted from the minimum PEF (morning or night-time) to obtain the amplitude (max-min), which is then divided by the mean value for the day and expressed as a percentage: % lability = (max-min)/mean x 100. A diurnal variation of 20% or more was a good indicator of asthma (Hetzel et al 1980). Enright et al (1994) recommended the upper limits of normal PEF lability in healthy non-asthmatic individuals, as determined by 95th percentiles, 31% in children 6 to 14 yr of age and 19% for adults.

There is no agreement on the best way to analyse the pattern of PEF changes. A commonly used method of expressing diurnal changes is to divide the difference between morning and evening values by their mean, averaged over a period of time (amplitude % mean). In order

not to lose isolated important drops in PEF, a method known as low percentage best, which is the lowest PEF as a percentage of the highest PEF occurring over a period of time, is also used. It is suggested that PEF variation as low % best is easy to perform and clinically relevant, especially in children (Brand et al 1997).

## 3. AIMS OF THE STUDY

1. To investigate the long-term pulmonary outcome, especially bronchial lability, in schoolchildren born very preterm with and without a history of CLD.

2. To assess whether neonatal surfactant therapy has had an effect on long-term pulmonary outcome in schoolchildren born very preterm.

3. To evaluate whether the abnormal lung function in schoolchildren born very preterm has any immunological features.

4. To evaluate the reproducibility of home monitoring of pulmonary function in children and the usefulness of this method for determining bronchial lability in children.

# 4. SUBJECT AND METHODS

## 4.1. Study populations

### Whole study population

The whole study population of 63 schoolchildren born very preterm (birth weight <1500 g and/or gestational age <30 wk) were part of a group of infants treated at the neonatal intensive care unit of the Children´s Hospital, University of Helsinki, Finland, between 1980 and 1987. Neonatal data were collected from the hospital records (Table 1). Eighteen (29%) of these children had a history of CLD (Shennan et al 1988). The children were subdivided into two groups according to growth status at birth: appropriately grown (AGA) and with intrauterine growth retardation (IUGR). For that purpose, birthweights and heights were transformed to gestational age-specific SD units by using fetal growth charts for Finnish children for girls and boys (Pihkala et al 1989). AGA was considered to be present when birth weights ranged from -2 to + 2 units. IUGR was diagnosed when the gender- and gestational age-specific birth weight was < -2 SD units. Eleven of the 63 children (11%) were IUGR. A group of 25 healthy, local schoolchildren of the same age, all of whom were born at term, were recruited as a control group. In the methodological part of the study 110 children, all born at term, with newly diagnosed asthma, were selected as our series for evaluating the reproducibility of home spirometry.

### Study populations in different publications

**Study I** was a cross-sectional study assessing lung function at school age in CLD and non-CLD groups. In study I the patients were part of a group of VLBW infants (<1250 g) treated at the neonatal intensive care unit of the Children´s Hospital, University of Helsinki, Finland, between 1980-1985. Among the total of 168 survivors, 70 children (42%) had required oxygen therapy for more than 28 days, and 22 children (13%) were still oxygen-dependent at the age of 36 postconceptional weeks, which was used as the criterion of CLD (Shennan et al 1990). Of the 22 children with histories of CLD, 12 were traced and were able to participate in our study. Their gestational ages at birth and the duration of oxygen therapy did not differ

significantly from those of the remaining CLD children who were not examined (median gestational age at birth 27.0 vs 26.5, median duration of oxygen therapy 80.5 vs 78.5 days). Twenty children without CLD but comparable in birth weight and gestational age were also included; 17 of them completed the study (Table 2). A group of 22 healthy, local schoolchildren of the same age, all of whom were born at term, were recruited as a control group for spirometry. Lung function of seven control children was recorded at home.

Table 1. Neonatal data of 63 schoolchildren born very preterm

| Characteristics | | CLD group N = 18 | Non-CLD group N = 45 | p values |
|---|---|---|---|---|
| Birth weight | g | 940 (595-1200) | 1100 (670-1730) | 0.001 |
| | SD | -0.60 (-4.1-2.40) | -0.20 (-4.9-1.9) | 0.43 |
| Birth height, | cm | 35.0 (30.0-37.5) | 36.0 (30.0-41.0) | 0.008 |
| | SD | -1.25 (-5.8-4.1) | -0.30 (-6.70-3.30) | 0.83 |
| Gestational age, wk | | 26.8 (24.1-30.1) | 27.9 (24.6-35.0) | 0.03 |
| Apgar score | 1 min | 3.0 (0-8) | 5.0 (1-10) | 0.05 |
| | 5 min | 6.5 (1-8) | 7.0 (2-10) | 0.05 |
| IMV, days | | 53 (6-165) | 5 (0-58) | < 0.0001 |
| $FiO_2 > 0.21$, days | | 74 (43-512) | 11 (1-65) | < 0.0001 |
| $FiO_2 > 0.4$, days | | 25 (2-257) | 1 (0-11) | < 0.0001 |
| Postconceptional age when $O_2$ discontinued. wk | | 39 (36.1-98.0) | 31 (27.5-35.6) | < 0.0001 |
| Boys | | 9 (50%) | 26 (58%) | 0.32 |
| RDS | | 17 (94%) | 30 (67%) | 0.02 |
| PDA | | 13 (72%) | 16 (36%) | 0.008 |
| Air leak | | 2 (11%) | 1 (2%) | 0.13 |
| Sepsis | | 3 (17%) | 4 (9%) | 0.37 |
| Tracheal/bronchial stenosis | | 5 (28%) | 1 (2%) | 0.002 |

IMV=intermittent mandatory ventilation, $FiO_2$=fraction of inspired oxygen
Values are expressed as medians (range) or as numbers (%) in the given category

Table 2. Neonatal data of children participating in study I

| Characteristics | CLD group N = 12 | Non-CLD group N = 17 | p values |
|---|---|---|---|
| Boys/girls | 4/8 | 7/10 | NS |
| Birth weight, g | 955 (620-1200) | 980 (690-1240) | NS |
| Gestational age, wk | 27 (24-30) | 28 (25-35) | NS |
| IMV, days | 53 (16-165) | 9 (0-58) | < 0.001 |
| $FiO_2$ > 0.21, days | 81 (50-135) | 35 (1-65) | < 0.0001 |
| $FiO_2$ > 0.4, days | 26 (5-87) | 1 (0-6) | < 0.0001 |
| Postconceptional age when $O_2$ discontinued, wk | 39 (36-44) | 34 (29-35) | < 0.0001 |
| RDS * | 10 (83%) | 9 (53%) | < 0.05 |
| PDA ** | 8 (67%) | 7 (41%) | NS |
| Air leak | 1 (8%) | 0 (0%) | NS |
| Sepsis | 2 (17%) | 2 (12%) | NS |
| Tracheal/bronchial stenosis | 4 (33%) | 1 (6%) | NS |

IMV=intermittent mandatory ventilation, $FiO_2$=fraction of inspired oxygen
Values expressed as medians (range) or as numbers (%) in given category
* Typical chest X-ray changes and requirement of supplementary oxygen for at least 2 days
**Requirement of pharmacological or surgical closure

**Study II** was a cross-sectional study evaluating the effect of surfactant therapy on lung function at school age. The series examined in study II consisted of patients who were born before 30 weeks of gestation and had participated in the following three randomized bicenter (University of California, San Diego, CA., and University of Helsinki, Finland) trials of human surfactant therapy in the Children´s Hospital, University of Helsinki, Finland, between 1983-1987 (Hallman et al 1985 (study 1), Merritt et al 1986 (study 2), Merritt et al 1991 (study 3)). In study 1, human surfactant was given to infants with established neonatal RDS (rescue therapy) after clinical, radiographic, and biochemical documentation of surfactant deficiency (Hallman et al 1985). In study 2, human surfactant was given within 10 minutes of birth to infants at high risk of neonatal RDS (Merritt et al 1986). Study 3 was a placebo-controlled comparison between prophylactic and rescue therapy of human surfactant given to small preterm infants with lung immaturity (Merritt et al 1991). Infants who were randomized to the placebo group received an injection of air from shielded syringes in an identical fashion to the surfactant-treated infants.

Of the Finnish children who participated in the trials during 1983-1987 in Helsinki, 65 survived the first year of life. The addresses of seven (11%) could not be traced, nine (14%)

refused to participate, and nine (14%) children were unable to perform the tests because of neurological limitations. Forty children completed the tests of pulmonary function at 7-12 years of age. Of them, 17 were originally included in the prophylactic group, 14 in the rescue group, and 9 in the placebo group. No statistically significant differences in gestational age, birth weight, duration of oxygen or ventilator treatment were observed between these three groups or between the three studies. The duration of high oxygen treatment tended to be shorter in the prophylactic group than in other groups (p=0.04). Eleven (28%) children dependent on supplementary oxygen at the age of 36 postconceptional weeks, were diagnosed as having CLD (Shennan et al 1988) (Table 3). The infants who did not participate in the follow-up study had similar incidence of CLD. Prenatal glucocorticoids were not used. Only three children had received glucocorticoid during treatment in the neonatal intensive care unit. As a reference group, we recruited 20 healthy, local school children of the same ages, all of whom were born at term. None of them had respiratory symptoms at the time of the study.

Table 3. Neonatal data of children participating in study II

| Characteristics | Prophylactic group N = 17 | Rescue group N = 14 | Placebo group N = 9 |
|---|---|---|---|
| Boys/girls | 14/3 | 8/6 | 4/5 |
| Birth weight, g | 1119 (258) | 1025 (316) | 1097 (282) |
| Gestational age, wk | 27.4 (1.6) | 27.1 (1.6) | 27.4 (1.4) |
| IMV, days | 7 (1-85) | 8 (1-165) | 14 (1-80) |
| $FiO_2 > 0.21$, days | 10 (3-131) | 49.5 (2-512) | 43 (1-75) |
| $FiO_2 > 0.4$, days | 1 (0-87)* | 3 (0-257) | 5 (0-45) |
| Postconceptional age when $O_2$ discontinued, wk | 29.9 (27.7-43) | 33.8 (27.5-98) | 34.0 (28-46.4) |
| Tracheal/bronchial stenosis | 1 (6%) | 2 (14%) | 0 (0%) |
| CLD ** | 2 (12%) | 5 (36%) | 4 (44%) |

Values are expressed as means (SD), as medians (range) or as numbers (%) in a given category. * Significant difference compared with the other groups (p=0.04).
** Requirement of supplementary $O_2$ at the age of 36 postconceptional weeks.

**Study III** was an immunological evaluation at school age in children born very preterm. This study was the follow-up study to investigate whether there had been an immunological basis for the lung function abnormalities in schoolchildren born very preterm, 34 (24 males and 10

females) of these 40 children in study II participated, at a median (range) age of 8.8 (7.4-11.7) years. The median (range) birth weight of these children was 1090 (595-1730) g and the median gestational age 27.4 (24.9-29.9) weeks. The median (range) duration of mechanical ventilation and duration of supplementary oxygen treatment during the neonatal period were 5.5 (1-129) and 10 (1-512) days, respectively.

A group of 14 healthy schoolchildren of the same age (median (range) age 9.1 (5.8-13) years), born at term, was recruited as a control group. These children were attending school at the time of the study and had neither respiratory symptoms nor symptoms of atopy.

**Study IV** was a longitudinal study evaluating the effect of inhaled budesonide on lung function of schoolchildren born very preterm. Twenty-one of all 63 children (studies I and II) born very preterm without a previous diagnosis of asthma, but with bronchial obstruction, increased responsiveness to a $beta_2$ agonist and/or abnormal diurnal PEF variation (i.e. $\geq$ 20%) were selected for the budesonide trial, which was completed by 18 children. The median (range) birth weight of these children was 1054 (640-1600) *g* and the median (range) gestational age 28 (24-35) weeks. The median (range) duration of mechanical ventilation and duration of supplementary oxygen treatment during the neonatal period were 6 (0-80) and 27 (1-131) days, respectively. Six children were dependent on supplementary oxygen at the age of 36 postconceptional weeks, which was used as the criterion of CLD (Shennan et al 1988). Nine had been treated with exogenous surfactant.

A group of 11 healthy local schoolchildren of the same age (mean (SD) age 10.3 (2.4) years), all born at term, were recruited as a control group for home monitoring. These children were attending school and had no respiratory symptoms at the time of the study.

**Study V** was a methodological study assessing the reproducibility of home monitoring in children. In study V, 110 outpatients, at a mean (SD) age of 7.4 (1.6) years, of whom 62% were male, with newly diagnosed asthma, were selected as our series for evaluating the reproducibility of home spirometry. The children participated in a study of early

pharmacological intervention in childhood asthma. Inclusion criteria were asthma symptoms, abnormal diurnal variation of PEF (≥20%) in the last 3 months preceding the first visit to the clinic, recorded daily at home in paper diaries; and/or a ≥ 15% change as measured by PEF or $FEV_1$ or ≥ 36% by $FEF_{50}$ proved either by a bronchodilator test or in an exercise test in the clinic or during home monitoring.

Before the studies, informed consent was obtained from all parents and children over 7 years old. All studies were approved by the Ethics Committee of the Department of Allergic Diseases at Helsinki University Central Hospital.

### 4.2. Study designs

#### Studies I-IV

For the lung function studies, all 63 schoolchildren born very preterm (studies I-IV) made two visits to the Outpatient Department of Allergic Diseases, in the University of Helsinki Central Hospital. These children underwent lung function testing, using an identical protocol and equipment.

At the first visit, clinical status was studied, and questionnaires focusing on respiratory symptoms during the preceding year were filled out. Flow-volume spirometry and a bronchodilator test were performed, and the children were trained to use a home spirometer. In addition, skin-prick tests were made. After the hospital visit, lung function was monitored twice daily at home for 4 weeks. The effect of a $\beta_2$ agonist was measured by spirometry before and after terbutaline inhalation every morning and evening for the first 2 weeks of home monitoring, followed by treatment with a placebo for 2 weeks. Parents and children were blinded to these treatments.

At the second visit, flow-volume spirometry was performed, and the data of the home spirometer were downloaded. The spirometric values measured at the first visit were used to evaluate basic lung function. The values at the second visit were used to compare spirometry

at home and in the clinic. In study I a histamine challenge test were performed and in study III a blood sample was taken for determination of serum ECP and MPO, and for measurement of lymphocyte subsets at the second visit.

Selected 18 children (study IV) were treated with inhaled budesonide (Pulmicort Turbuhaler$^{R}$, 0.2 mg/dose, Astra Draco AB, Lund, Sweden) in initially high (0.8 mg/m$^2$/day for 1 month) and subsequently lower doses (0.4 mg/m$^2$/day for 3 months) divided into morning and evening doses. Monitoring of spirometric values at home continued twice daily for 4 weeks during the first month of budesonide treatment. After that (at the third visit to the clinic), flow-volume spirometry was performed and the home spirometer was downloaded. During the last 4 weeks of the GC treatment period, home recording of spirometric values was performed twice daily. At the fourth visit to the clinic, body height and weight were measured, flow volume spirometry was performed, and the home spirometer was downloaded. The mean FVC, $FEV_1$ and PEF values during the second week of the $\beta_2$ agonist, a placebo and GC treatment were calculated. The numbers of abnormal diurnal PEF variations were recorded and compared between the following periods: 2 weeks of terbutaline treatment, 2 weeks of placebo treatment, and 2 weeks of GC treatment, respectively.

**Study V**

In study V, the 110 children born at term with newly diagnosed asthma made two visits to the Outpatient Department of Allergic Diseases, Helsinki University Central Hospital, for the lung function studies. Home spirometry was performed during the run-in phase of a study of early pharmacological intervention in childhood asthma. Each child underwent a lung function recording. The protocol and equipment used were identical throughout the study. At the first visit to the clinic, a physical examination was performed and body height and weight were measured. The children were instructed step by step in the use of the home spirometer. Lung function was then monitored twice daily at home for 24 days (mean) before starting the anti-inflammatory asthma medication. During the run-in period, the children were allowed to use an inhaled $\beta_2$ agonist if needed. Measurements with the spirometer was recorded at home every morning between 0600 and 1000 hours and every evening between 1800 and 2200

hours. Flow-volume spirometry was performed at the first and second visits. At the second visit to the clinic, the data of the home spirometry were downloaded on a computer.

All 25 control children underwent lung function testing, using a protocol and equipment identical to that used for the study children. Spirometry was done by all the control children, a group of 14 children underwent the histamine challenge test and 11 were recruited as a control group for the home monitoring.

### 4.3. Skin prick tests

Skin tests were performed by a trained nurse at the Outpatient Department of Allergic Diseases, Helsinki University Central Hospital, with the skin-prick technique, using eight major respiratory allergen extracts. A negative control solution and histamine hydrochloride (10 mg/ml) as positive control were used. Atopy was defined as at least one wheal $\geq$ 3 mm in diameter as a reaction to an allergen in the absence of a response to the negative control solution. Familial atopy was defined as physician-diagnosed allergy among first-degree relatives. Atopy of control children was evaluated by any evidence of specific serum IgE (positive Phadiatop Combi test$^{R}$, Pharmacia Diagnostics, Uppsala, Sweden).

### 4.4. Lung function and the nonspecific bronchial provocation tests

Flow volume spirometry was done with a pneumotachograph (Spirotrac III$^{R}$, Vitalograph Ltd., Buckingham, UK). At least three technically correct forced expiratory curves should be made according to the acceptability criteria of the American Thoracic Society (ATS). The curve was regarded as reproducible if the largest FVC and $FEV_1$ and second largest FVC and $FEV_1$ of the acceptable curves did not vary by more than 5% of the reading (expressed as a percentage of the largest observed FVC and $FEV_1$, regardless of the curve on which it occurred) (ATS 1995). The curve with the highest sum of $FEV_1$ and FVC values was selected for statistical analysis. Results of lung-function tests were analysed as percentages of predicted values, according to Polgar and Promadhat (Polgar and Promadhat 1971). The following spirometric parameters were recorded: FVC, $FEV_1$, $FEV_1$/FVC ratio, PEF, $FEF_{50}$

and $FEF_{75}$. Bronchial obstruction was defined as at least two of the following spirometric values: $FEV_1$<80%, PEF<75% or $FEF_{50}$<62% (Polgar and Promadhat 1971).
In the bronchodilator test in the office, flow-volume spirometry was measured before and 15 min after inhalation of terbutaline 0.5 mg from a dry powder inhaler (terbutaline sulfate 0.25mg/dose, Bricanyl Turbuhaler$^R$, Astra Draco AB, Lund, Sweden). During each terbutaline inspiration, peak inspiratory flow through the dry powder inhaler, Turbuhaler, with a pneumotachograph (Spirotrac III$^R$) was recorded ($PIF_{TBH}$). The lowest acceptable $PIF_{TBH}$ value was 30 l/min (Pedersen et al 1990). The changes in $FEV_1$ and PEF values after terbutaline were expressed as the differences (%) between the values before and after terbutaline inhalation ($\Delta FEV_1$ and $\Delta PEF$). A positive response to terbutaline was defined as a $FEV_1$ or PEF increment of $\geq$ 15% after terbutaline in the clinic or at home (ATS 1991).

At home, lung function was recorded with a Vitalograph Data Storage Spirometer (Vitalograph) particularly designed for long-term recording and storage of lung function parameters. The device consists of a pneumotachograph and a built-in electronic diary. Before use, the device was calibrated with a standard volume (variation within +/- 1%). After home recordings, the calibration was rechecked. The difference between these two calibration values (before and after home monitoring) was calculated. At home, FVC, $FEV_1$ and PEF values were recorded and the date and time logged at each test session. The child should stand up during the procedure and use a nose clip. The test was repeated a maximum of five times, until the results of the two best values met the reproducibility criteria of ATS. If this was not achieved with five tests, failure was recorded. The spirometer stored the curve with the largest sum of $FEV_1$ and FVC in the built-in electronic diary. The measurements were made at least 5 hours after inhalation of a $\beta_2$ agonist. The compliance of inhalation therapy was recorded by measuring $PIF_{TBH}$. During the first 2 weeks of home monitoring, the children inhaled terbutaline 0.25 mg twice daily. They performed spirometry every morning and evening before and 15 min after terbutaline inhalation, followed by treatment with a placebo for 2 weeks. The data from the home spirometer were analysed after 4 weeks´ monitoring. The number of PEF increments reaching 15% or more after terbutaline was used for the analysis (ATS 1991). The diurnal PEF variation of the whole 4 weeks´ recordings was calculated as the difference between the morning and evening PEF values and expressed as a percentage of

the greater PEF value. The number of ΔPEF ≥ 20% was used in the analysis. ΔPEF ≥ 20% has been shown to be a good indicator of bronchial lability (Hetzel et al 1980, Lebowitch et al 1991). The number of positive terbutaline responses and the number of ΔPEF ≥ 20% were expressed as percentages of all the tests during home monitoring. PEF increments ≥ 15% after terbutaline at least three times (≥ 11%, expressed as percentages of all the tests during home monitoring) during 2 weeks and diurnal PEF variation ≥ 20% at least four times (≥ 14% of all tests) during 4 weeks were considered abnormal. The mean $FEV_1$, FVC and PEF values, defined as the means of the morning values during the last week of home monitoring periods, were calculated.

In study V the reproducible home measurements were calculated as percentages of all the spirometric tests registered in order to evaluate the reproducibility of home spirometry. We also calculated the home spirometry measurements according to looser criteria than those set by ATS, i.e., accepting levels at which the largest and second largest FVC and $FEV_1$ values from reproducible spirometric curves at home did not differ by ≥ 10% or ≥ 15% of the recordings. In order to evaluate the compliance in home monitoring, we calculated the number of computer-registered spirometric tests and expressed the result as a percentage of the number of all desired tests during the whole home monitoring period.

In study I the histamine challenge test followed a protocol previously described (Sovijärvi et al 1993). Airway responsiveness to histamine, expressed as the histamine dose (mg) resulting in a 15% decrease in $FEV_1$ ($PD_{15}$ $FEV_1$), was determined using an automatic, inhalation synchronized, dosimetric jet nebulizer (Spira Elektro 2, Respiratory Care Centre, Hämeenlinna, Finland). The device incorporates a turbine flow sensor for monitoring tidal volume and inspiratory flow. With a two-bar driving pressure, the airflow to the nebulizer is 7.5 l/min. The nebulization time is 0.4 s, set to start at 100 ml after the beginnig of inspiration. During the test, the child is breathing with a tidal volume of 0.3-0.5 litres. The peak inspiratory flow is not allowed to exceed 0.5 l/s during administration of the aerosol. One, four, eight and sixteen tidal inspirations of buffered histamine diphosphate in saline solution with concentrations of 4 and 16 mg/ml are employed using a four-step dosage scheme. The calculated non-cumulative doses of histamine to the lungs and airways at each

step are 0.025, 0.1, 0.4, 0.8 and 1.6 mg, respectively. The $FEV_1$ measured with a pneumotachograph is used to determine the response to histamine. The $PD_{15}FEV_1$ is determined by manually plotting the dose of histamine against the % fall in $FEV_1$ using a logarithmic scale for the histamine doses. The $PD_{15}FEV_1$ is obtained by linear interpolation between the last two points. $PD_{15}$ $FEV_1$ values $\geq$ 1.6 mg histamine are considered normal for adults (Sovijärvi et al 1993). After the last histamine dose, 0.5 mg terbutaline was given. Terbutaline was inhaled from a metered-dose inhaler with a valved spacer device (Nebuhaler$^R$, Astra Draco AB, Lund, Sweden). If the child had a respiratory infection, the test was not performed until 2 weeks after the infection had ended. The children were not allowed to use $\beta_2$ agonists for at least 12 h before the challenge test.

### 4.5. Markers of inflammation

In study III, blood for ECP and MPO was taken in gel tubes (SST, Becton-Dickinson, England) and allowed to clot at room temperature for 60 (±10) min. Serum was separated by centrifugation at 1350 x g for 10 min and stored at -20$^o$ C until analysed for ECP (Venge et al 1977) and MPO (Bousquet et al 1991), using a radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer´s instructions. Blood eosinophils were counted with a haematological analyser (Advia 120, Bayer).

For flow cytometric analysis, peripheral venous blood samples were drawn in heparin. Thereafter, mononuclear leucocytes were isolated by Ficoll-Paque centrifugation at 400 g for 30 min. Subsequently, a mononuclear blood cell suspension at a cell concentration of 1 x $10^6$/ml in RPMI 1640, with antibiotics, glutamine, and 5% fetal calf serum, was incubated at $4^0$ C for 10 min with monoclonal antibodies against CD3, CD4, CD8, CD19, CD23, CD25 and CD16+56 (which are natural killer (NK) cells) (Beckton Dickinson Immunocytometry Systems, Mountain View, California) according to the manufacturer´s instructions (Macey 1994). Flow cytometric analysis was then performed, using the same instrument settings in each analysis. The lymphocytes were finally gated to study the numbers of cell subsets within the gate. The numbers of cells positive for the surface markers are expressed as percentages of all the cells within the gate.

### 4.6. Statistical methods

Statistical analyses were performed using the Statgraphics computer program (studies I, II, IV), the MacIntosh Stat View computer program (study III) and the statistical package STATISTICA (release 5.0 for Windows) (study V and combined data). Results of lung function tests were analysed as percentages of predicted values (Polgar and Promadhat 1971).

In study I the significances of the differences between the three study groups were tested using one-way analysis of variance for normally distributed data. A Kruskal-Wallis non-parametric test was used if the distribution was not normal or if the variances were not homogeneous. Fisher´s exact test and the Mann-Whitney test were used for assessing the significance of differences between the two groups. Spearman´s rank correlation ($r_s$) was used in analysis for the association between current symptoms and the lung function parameters. Multiple stepwise regression analysis was used to evaluate the relationship between the neonatal (independent variable) and current pulmonary function (dependent variable) data. The following neonatal variables were evaluated: birth weight, duration of oxygen therapy ($FiO_2$>0.40 and $FiO_2$>0.21), duration of ventilator treatment, postconceptional age when $O_2$ discontinued, and history of tracheal/bronchial stenosis.

In study II the study groups were tested for significant differences using one-way analysis of variance for normally distributed data. A Kruskal-Wallis non-parametric test was used if the distribution was not normal or if the variances were not homogeneous. Fisher´s exact test was used for assessing the significance of differences between the two groups. Multiple regression analysis was used for evaluating the relationship between the prenatal-neonatal variables and the pulmonary function data. On the basis of the correlation matrix consisting of 38 neonatal variables and the lung function parameters, the following two independent variables were evaluated for association with the spirometric parameters: surfactant treatment (yes/no) and the duration of intubation. In addition, the following variables were evaluated: duration of $O_2$ therapy of at least 40%, duration of supplemental $O_2$ therapy, and diagnosis of tracheobronchial stenosis (yes/no) and of CLD. Being associated with CLD and/or fatal neonatal RDS, the SP-A/DPC ratio, analysed in airway specimens recovered at the age of one

day, was used as the covariate. Gestational age was included as another covariate in the regression analysis.

In study III the data of the children born very preterm and the controls were compared using the Mann-Whitney test or the Fisher exact test. A Kruskal-Wallis non-parametric test was used to determine the differences between the three study groups (preterm-born schoolchildren with and without bronchial obstruction and controls). Spearman´s rank correlation ($r_s$) was used to evaluate the association between lung function and the results of the blood tests overall and within the groups.

In study IV, Spearman´s rank correlation ($r_s$) was used to analyse the correlation between the neonatal variables and the subsequent lung function parameters and between the different lung function parameters and the symptoms. Statistical comparisons of longitudinal lung function measurements during terbutaline, placebo and budesonide treatment were performed with Friedman´s non-parametric analysis of variance.

In study V the reproducibility of the home measurements was estimated by calculating the percentage of home spirometry measurements that met the levels of reproducibility $\leq$ 5% (ATS criteria), <10% and <15%. The children were assigned to three categories according to age (5-6 years, 7-8 years and 9-10 years), and to height (< 121 cm, 121-135 cm and 136-150 cm). Differences between groups were tested using analysis of variance (ANOVA), and, to adjust for multiplicity, Fisher`s LSD method was used in pairwise comparisons. The differences between compliance in performing the spirometric tests and the reproducibility of the tests during the first and third week at home were analysed by the paired t-test. Regression analysis was used to evaluate the relationship between age and height and the percentage of home spirometry measurements that met the ATS criteria. Pearson correlation coefficients were calculated to study the relationship between age and height versus the percentage of reproducible home spirometry measurements, using three levels of reproducibility, $\leq$ 5 %, <10 % and <15 % and also between baseline spirometry in the clinic and the percentage of home spirometry measurements that met the ATS criteria.

In combined data (Tables 1, 4, 5, 6 and 7) t-test for independent samples was used to compare the CLD group with the non-CLD group. In a case of skew variables, the Mann-Whitney test was used. The chi-square test was used to compare binary variables. Analysis of variance was used to compare more than two groups and, because of non-equal variances, Games-Howell tests were performed for post hoc multiple comparisons. Kruskal-Wallis and Mann-Whitney tests for pairwise comparisons were done if the normal distribution assumptions could not be made. Stepwise regression analysis was performed to identify the best combinations of neonatal variables to predict the current pulmonary function variables, and correspondingly stepwise logistic regression analysis was performed to identify the explaining variables in binary conditions, such as bronchial obstruction. In analysing the association between the different lung function parameters, Spearman´s rank correlation ($r_s$) was used.

# RESULTS

## 5.1. Bronchial lability and responsiveness in schoolchildren born very preterm (Study I and study II)

*Patients*

In all 63 children, age, height in SD units, weight, and weight expressed as a percentage of the mean weight of children of the same sex and height (Sorva et al 1984) did not differ between the CLD, non-CLD and control groups. Heights as SD in relation to age were significantly lower in the CLD children than in the full-term controls (p=0.02) (Table 4).

*History of respiratory symptoms and atopy*

A total of 38 children (60%) had had respiratory symptoms during the year prior to the study. Twenty-six children (41%) had had dyspnoeic symptoms at least once and, in addition, 12 children had suffered from continuous coughing for more than 3 weeks. Ten children (16%) had asthma diagnosed by a physician. Eight of them were receiving continuous budesonide or disodium cromoglycate inhalation therapy and one child used a $\beta_2$ agonist during infections. The groups did not differ in individual or familial atopy or in parental smoking. Atopy was found in 28% of patients in the CLD and in 18% in the non-CLD group. The respective percentages of familial atopy were 44 vs. 36%. The percentages of parental smoking were high in both groups; 39% in the CLD and 40% in the non-CLD group (Table 5).

*Basic lung function*

All 63 children performed a complete set of spirometer tests (three pre- and three post-$\beta_2$ agonist) in the clinic. Fifteen children (83%) in the CLD group and fourteen (31%) in the non-CLD group had spirometric values in the clinic indicating bronchial obstruction ($p<0.001$) (Table 5). Of these children with obstruction, 18 (62%) reported respiratory symptoms during the past year. All spirometric values were significantly lower in the preterm group than in the full-term controls ($p \leq 0.01$). When the three groups were analysed separately, all spirometric values except FVC in the non-CLD group were

Table 4. Demographic data at entry of 63 schoolchildren born very preterm

| | CLD group N = 18 | Non-CLD group N = 45 | Full-term N = 25 | p values ANOVA |
|---|---|---|---|---|
| Age, yrs | 9.8 (7.7-13.9) | 10.2 (7.4-12.8) | 10.6 (5.7-13.6) | 0.70 |
| Height, cm | 130.5 (114.5-150.5) | 135.7 (115.2-167.0) | 141.3 (115.1-165.1) | 0.14 |
| Height, SD units | -1.4 (-4.0 to 1.6)* | -0.5 (-2.2 to 2.8)** | 0 (-3.0 to 1.3) | p=0.03 |
| Weight, kg | 26.9 (16.5-45.0) | 30.8 (19.3-55.0) | 33.8 (17.0-67.3) | 0.13 |
| Height-related weight (%) | -1.0 (-20 to 17) | 0 (-27 to 55.0) | 0 (-16 to 54) | 0.76 |

Values expressed as medians (range)
* p = 0.02 compared with the full-term controls
** p =0.02 compared with the CLD group

Table 5. Respiratory symptoms, atopy, risk factors during the past year and present findings of bronchial obstruction

| Characteristics | CLD group N=18 | Non-CLD group N=45 | p values |
|---|---|---|---|
| Atopy | 5 (28%) | 8 (18%) | NS |
| Familial atopy* | 8 (44%) | 16 (36%) | NS |
| Parental smoking | 7 (39%) | 18 (40%) | NS |
| Physician-diagnosed asthma | 5 (28%) | 5 (11%) | NS |
| Respiratory symptoms | 12 (67%) | 26 (58%) | NS |
| Dyspnoeic symptoms | 9 (50%) | 17 (38%) | |
| Prolonged cough | 3 (17%) | 9 (20%) | |
| Bronchial obstruction | 15 (83%) | 14 (31%) | <0.001 |
| Bronchial lability** | 8 (47%) | 23 (54%) | NS |

* Physician-diagnosed allergy among first-degree relatives.
**A significant response in the bronchodilator test and/or abnormal diurnal PEF variation

significantly lower in both preterm groups than in the full-term controls (Figure 1). All spirometric values except $FEV_1$/FVC were significantly lower in the CLD than in the non-CLD group (Figure 1).

There were no significant differences in lung function between the children without a history of RDS and CLD (N=15) and those with a history of RDS but not CLD (N=30) when the groups were analysed separately. However, the children without a history of RDS and CLD had significantly lower $FEV_1$, PEF, $FEF_{50}$ and $FEF_{75}$ values than the healthy controls (Table 6, Figure 2).

*Bronchodilator test and diurnal PEF variation*

In the bronchodilator test in the clinic, one of the study children had a significant response to terbutaline ($\Delta FEV_1 \geq 15\%$) (ATS 1991). Five children had $\Delta FEV_1 \geq 10\%$ (one non-CLD and four CLD children). There was no significant difference between the CLD and non-CLD groups; the median $\Delta FEV_1$ was 5.8% (range -5.1-29.1.3%) in the CLD and 2.3% (range -4.1-14.7%) in the non-CLD group (p=0.08).

During the 2 weeks´ recording at home, PEF increments ≥ 15% after terbutaline were observed at least three times (≥ 11%, expressed as percentages of all the tests during home monitoring) in 40% of the patients. According to this definition, none of the 11 control children had a positive bronchodilator test at home. The difference between the preterm group and the healthy controls were significant (p=0.002) (Figure 3). When the groups were analysed separately, there were no significant difference between the preterm born groups, although CLD and non-CLD groups differed significantly from the controls ($p \leq 0.01$).

Fourteen children (22%) had diurnal PEF variation ≥ 20% at least four times (≥ 14% of all tests) during home monitoring. According to this definition, none of the control children had abnormal diurnal PEF variation and the difference between the preterm group and the healthy controls was significant (p=0.006) (Figure 3). When the groups were analysed separately, there were no significant difference between the preterm born groups, although CLD and non-CLD groups differed significantly from the controls ($p \leq 0.03$).

Table 6. Lung function in three groups of schoolchildren born very preterm (according to CLD and RDS) and in full-term controls

| | A1<br>Non-RDS<br>Non-CLD<br>N = 15 | A2<br>RDS<br>Non-CLD<br>N = 30 | A3<br><br>CLD<br>N = 18 | B<br><br>Full-term<br>N = 25 | p<br>values<br>AI vs A2 | A2 vs A3 | A1 vs B |
|---|---|---|---|---|---|---|---|
| $FEV_1$ | 84.4 (39.3-103.8) | 87.3 (70.2-104.8) | 71.4 (40.7-88.9) | 94.2 (79.2-117.7) | 0.54 | <0.0001 | 0.04 |
| FVC | 97.0 (39.2-121.4) | 95.5 (76.5-114.6) | 84.6 (44.9-98.6) | 98.6 (84.0-123.7) | 0.99 | 0.01 | 0.75 |
| $FEV_1$/FVC | 89.3 (72.6-100.4) | 91.0 (77.9-109.3) | 87.3 (67.2-107.3) | 96.9 (85.9-105.4) | 0.23 | 0.34 | 0.008 |
| PEF | 73.8 (38.0-90.3) | 76.6 (53.6-100.7) | 59.1 (373-86.4) | 89.0 (72.5-110.9) | 0.48 | 0.001 | 0.002 |
| $FEF_{50}$ | 75.2 (37.4-102.9) | 84.4 (47.5-115.5) | 58.1 (29.5-89.5) | 108.4 (72.8-153.9) | 0.20 | 0.0002 | <0.0001 |
| $FEF_{75}$ | 56.9 (217-86.0) | 67.4 (35.3-137.4) | 45.0 (14.4-82.5) | 84.0 (51.6-146.6) | 0.13 | 0.006 | 0.0003 |
| Diurnal PEF variation ≥ 20% | | | | | | | |
| | N = 14 | N = 28 | N = 17 | N= 11 | | | |
| | 1.00(0-9) | 1.00(0-9) | 2.00(0-8) | 0.00 (0-1) | 0.54 | 0.22 | 0.07 |
| Bronchodilator test at home | | | | | | | |
| | 2.50(0-10) | 2.00(0-8) | 2.00(0-7) | 0.00(0-2) | 0.31 | 0.37 | 0.001 |

Values are expressed as medians (range).
Lung function tests are expressed as percentages of the predictive values or as numbers in a given category.

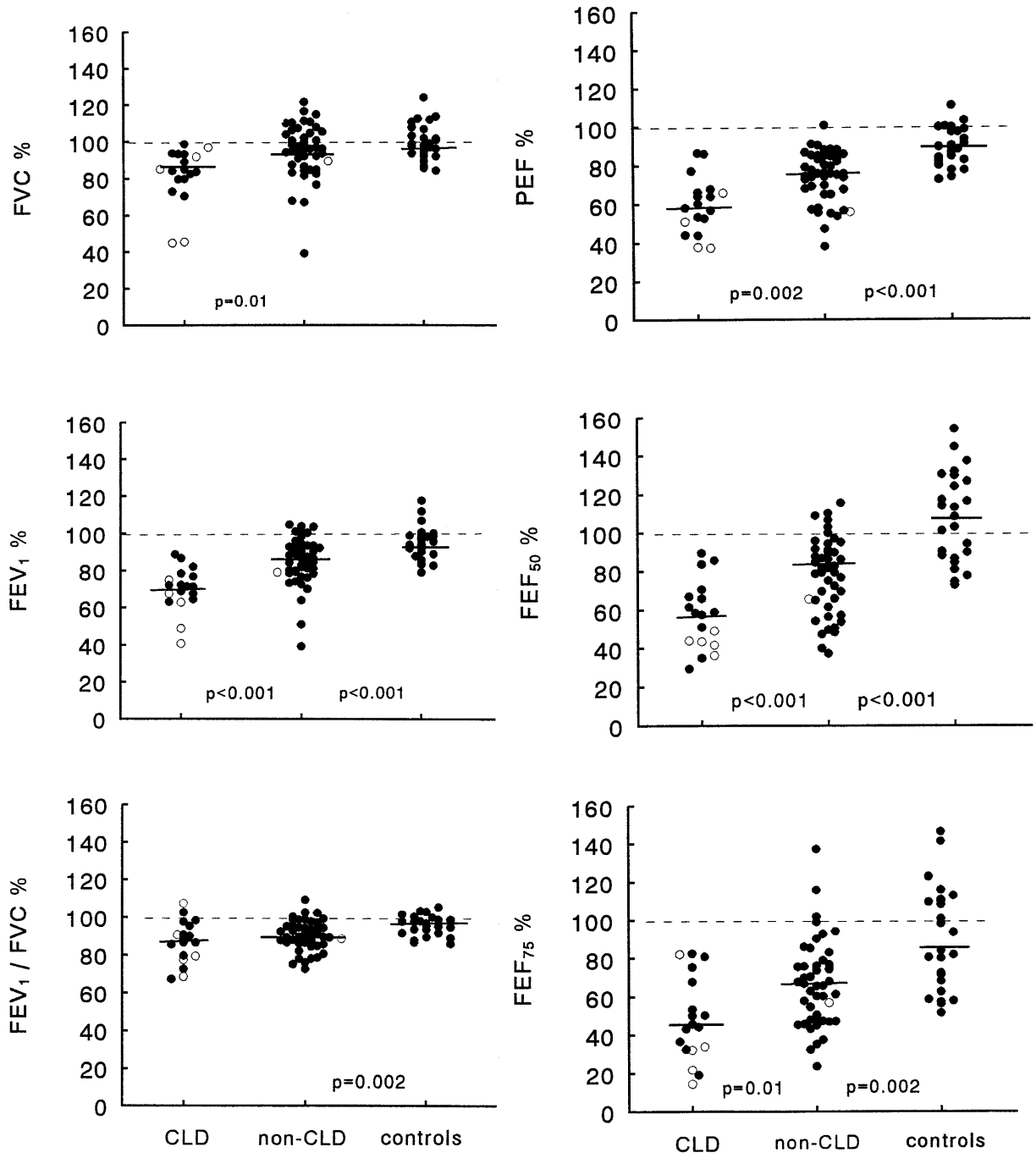


Figure 1. $FEV_1$, FVC and $FEV_1$/FVC (A) and PEF, $FEF_{50}$ and $FEF_{75}$ (B) values as percentages of predicted values of the children in the different study groups (open circles: history of tracheal/bronchial stenosis). Medians are indicated by horizontal lines. Significant differences are indicated in the figure when the three groups are analysed separately.

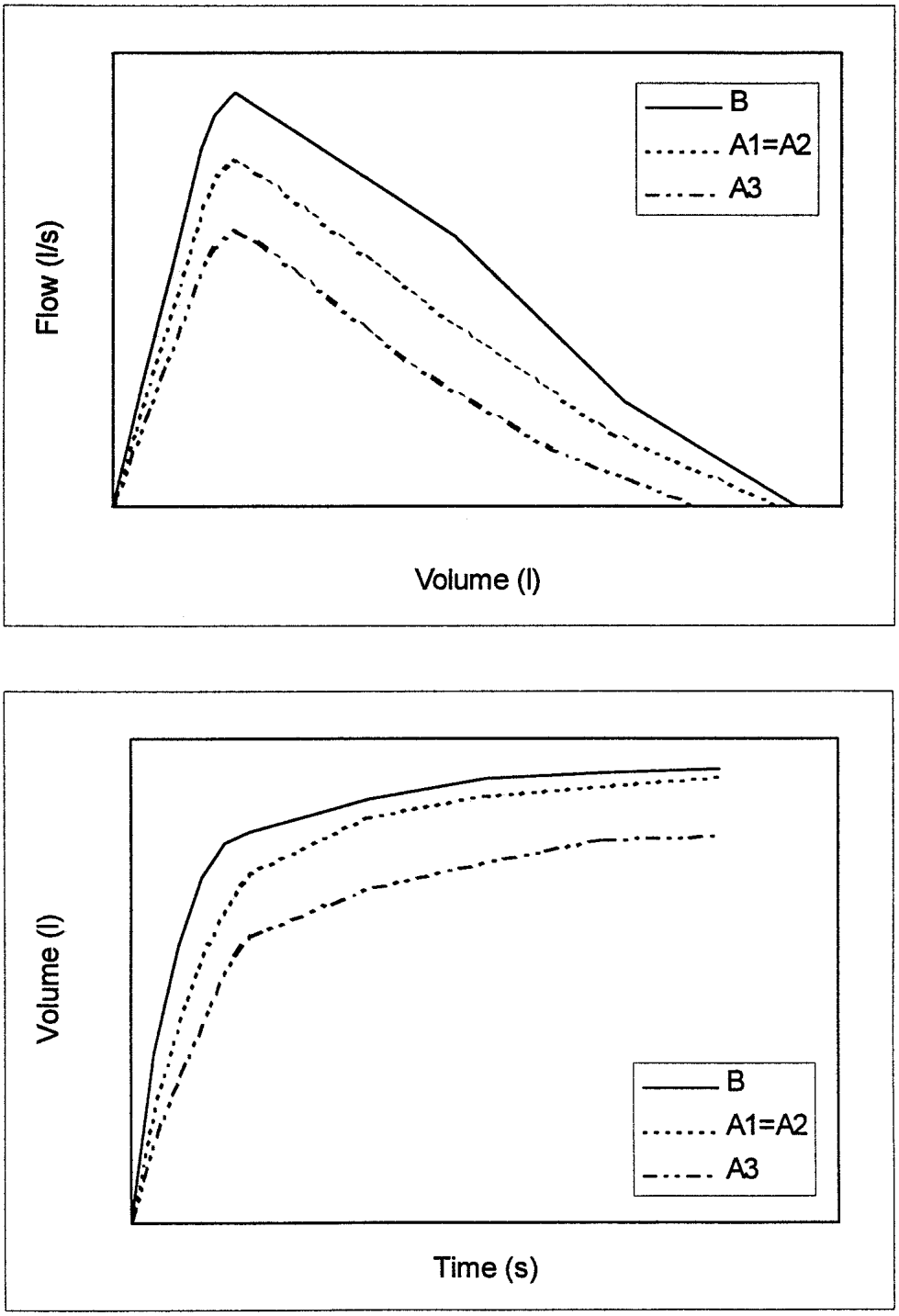


Figure 2. Schematic presentation of spirometric values measured in the clinic in the schoolchildren born very preterm and grouped according to the neonatal history. Flow-volume curves are presented above and time-volume curves below. A1; children born very preterm without a history of RDS and CLD (N=15). A2; children born very preterm with a history of RDS but without a history of CLD (N=30). A3; children born very preterm with a history of CLD (N=18). B; full-term controls (N=25).

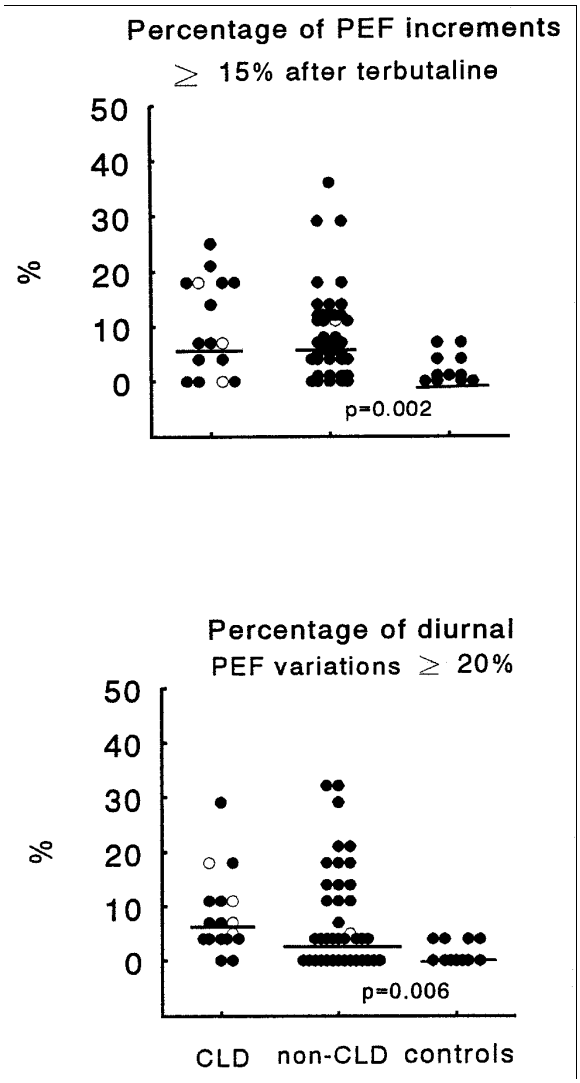


Figure 3. The number of PEF measurements with an increment ≥ 15% after terbutaline and diurnal PEF variations ≥ 20% during home monitoring expressed as percentages of all the tests during home monitoring (open circles: history of tracheal/bronchial stenosis). Medians are expressed as horizontal lines. Significant differences between the preterm group and the healthy controls are expressed in the figure. CLD: schoolchildren born very preterm with a history of CLD. Non-CLD: schoolchildren born very preterm without a history of CLD. Controls: full-term healthy controls.

A significant response in the bronchodilator test and/or abnormal diurnal PEF variation were observed in 30 of the 63 children tested (48%) and in 19 (66%) of the 29 children with verified obstruction. In the children with bronchial lability (a significant response in the bronchodilator test and/or abnormal diurnal PEF variation) only the PEF values were significantly lower than in children without bronchial lability (p=0.02). Significantly more of the preterm born children without a history of RDS and CLD had bronchial lability than of the healthy controls (Table 6).

*Bronchial responsiveness*

Median histamine $PD_{15}FEV_1$ was significantly lower in the CLD group than in the non-CLD group; 0.8 (range 0.06 - >1.6) vs. >1.6 (range 0.44 - >1.6), (p = 0.01) or in the full-term controls (>1.6, range 0.41- >1.6) (p=0.02). No significant differences were observed between the non-CLD group and the full-term controls (median $PD_{15}FEV_1$ >1.6 vs. >1.6 mg) (Figure 4).

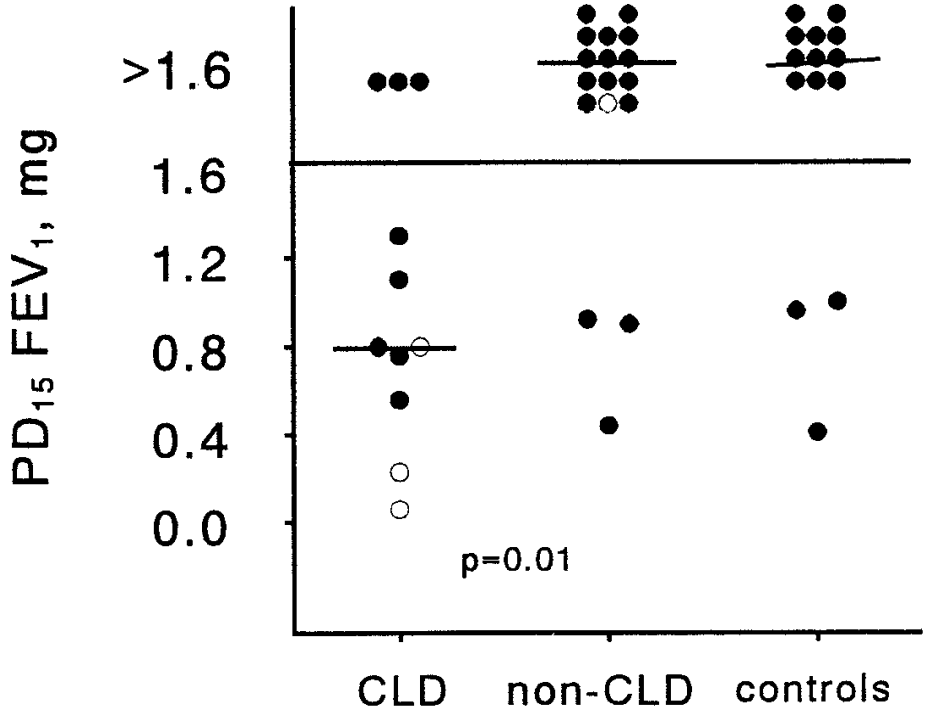


Figure 4. The results of histamine challenge tests as $PD_{15}FEV_1$ in the children in the different study groups (open circles: history of tracheal/bronchial stenosis). Medians are indicated by horizontal lines. Significant difference between the CLD group and the non-CLD group is indicated in the figure. CLD: schoolchildren born very preterm with a history of CLD. Non-CLD: schoolchildren born very preterm without a history of CLD. Controls: full-term healthy controls.

Home monitoring was not acceptable in three children because of technical faults in the device and one child with the history of tracheal stenosis had severe symptoms and was unable to perform the histamine challenge test or carry out home monitoring. With the other children the percentages of acceptable individual measurements at home was high: 89% (range 54-99%).

*Association of neonatal variables and current respiratory symptoms with subsequent lung function (all study children)*

The duration of ventilator treatment was significantly associated with FVC, PEF and $PD_{15}FEV_1$ values and the duration of oxygen therapy with $FEV_1$ and $FEF_{75}$ values. A history of tracheal/bronchial stenosis was associated with $FEF_{50}$. Parental smoking was associated with $FEV_1$ and PEF values (Table 7). Neither gestational age nor birthweight was associated with the spirometric data or with the bronchial responsiveness.

Table 7. Association between neonatal variables and subsequent lung function in schoolchildren born very preterm. Results of stepwise multiple regression analysis and logistic regression analysis:

| Dependent lung function variable | Independent variable | Regression coefficient | Standard error | p values | $R^2$ values (adjusted) |
|---|---|---|---|---|---|
| FVC | IMV (days) | -0.14 | 0.07 | 0.039 | |
| | Postconceptional age when $O_2$ discontinued (wk) | -0.50 | 0.24 | 0.040 | 0.331 |
| | Parental smoking* | -6.22 | 3.44 | 0.076 | |
| $FEV_1$ | IMV (days) | -0.12 | 0.06 | 0.067 | |
| | Postconceptional age when $O_2$ discontinued (wk) | -0.49 | 0.22 | 0.033 | 0.321 |
| | Parental smoking* | -6.51 | 3.23 | 0.049 | |
| PEF | IMV (days) | -0.19 | 0.05 | <0.001 | |
| | Parental smoking* | -9.5 | 3.4 | 0.007 | 0.290 |
| $FEF_{50}$ | $FiO_2$>0.4 (days) | -0.08 | 0.20 | 0.673 | |
| | Tracheal/bronchial stenosis (yes/no)* | -20.88 | 10.2 | 0.046 | |
| | Parental smoking* | -7.81 | 5.44 | 0.157 | 0.134 |
| | Postconceptional age when $O_2$ discontinued (wk) | -0.01 | 0.75 | 0.992 | |
| $FEF_{75}$ | $FiO_2$>0.4 (days) | -0.212 | 0.09 | 0.024 | |
| | Tracheal/bronchial stenosis (yes/no)* | -11.24 | 10.87 | 0.306 | 0.132 |
| $PD_{15}FEV_1$** | IMV (days) | 0.040 | 0.018 | 0.026 | |

IMV=intermittent mandatory ventilation, $FiO_2$=fraction of inspired oxygen
*No=0, yes=1
**Results are from logistic regression analysis. Odds ratio is 1.041 (95% confidence interval for odds ratio is 1.005 to 1.078). Positive=1, negative=0.

According to Spearman´s rank correlation test, $PD_{15}FEV_1$ values correlated with $FEV_1$, $FEV_1$/FVC, $FEF_{50}$, $FEF_{75}$ and PEF values measured in the clinic (range of r = 0.36 - -0.58; $p<0.05$ for all). Diurnal PEF variation correlated inversely with $FEV_1$, FVC, $FEF_{50}$ and PEF values measured in the clinic (range of r = -0.36- -0.42; $p<0.05$ for all). A bronchodilator response at home was inversely correlated with $FEV_1$, $FEV_1$/FVC, $FEF_{50}$, $FEF_{75}$ and PEF values in the clinic (range of r = -0.32 - -0.41; $p<0.05$ for all) and in the clinic with $FEF_{50}$ and $FEF_{75}$ values (range of r = -0.27 - -0.33; $p<0.05$ for both). According to Pearson´s correlation, there were no significant correlations between birth weight in SD units and lung function parameters.

In the group of schoolchildren born very preterm with respiratory symptoms, $PD_{15}FEV_1$ values tended to be lower than in children with no abnormal respiratory symptoms (p=0.07). Other lung function values were similar in these two groups. Atopy had no effect on lung function variables at school age. In the group of schoolchildren born very preterm whose parent(s) smoked, PEF values in the clinic were significantly lower than in the group with non-smoking parents (p=0.005). $FEV_1$ and FVC values in the clinic tended to be correspondingly lower (p=0.06 and p=0.08). In logistic regression analysis, bronchial obstruction at school age was significantly associated with CLD and with parental smoking, respectively (risk ratio 2.58, p=0.0002 and risk ratio 1.70, p=0.02).

## 5.2. Effect of neonatal surfactant therapy on lung function at school age in children born very preterm (Study II)

The ages of the children in the three different study groups (prophylactic, rescue and placebo groups) were similar. The children in the rescue group were significantly shorter than the controls, but between the other two groups there were no differences in height or weight. No significant differences in the prevalence of atopy or parental smoking were observed between the groups.

Of the spirometric values, FVC and PEF were significantly lower among the children in the placebo group than in any of the surfactant-treated children ($p<0.05$). The same parameters

were significantly lower in the placebo group than in the prophylactic surfactant group ($p<0.05$) (Figure 5). There were no significant differences in spirometric parameters between the prophylactic and the rescue groups, or between the patients in the three trials.

All spirometric parameters (FVC, $FEV_1$, PEF and $FEF_{50}$) were significantly lower in the preterm cohort than in the controls ($p<0.05$), except FVC in the prophylactic group. As compared with the values for the controls born at term and for the children with or without a history of CLD, only the FVC values for the children without CLD were not significantly reduced. All the other spirometric parameters were significantly lower in the preterm study groups ($p<0.001$) (Figure 5). There was no significant difference between the groups in $\Delta FEV_1$: the median $\Delta FEV_1$ was 3.3% (-4.1 to 15.0%) in the prophylactic group, 5.0% (-3.8 to 29.1%) in the rescue group and 1.9% (-3.0 to 14.7%) in the placebo group. During the 2 weeks of home spirometry monitoring, altogether 16 children (40%) had at least three positive PEF responses to terbutaline (p between groups NS). Eight children (20%) had abnormal diurnal PEF variation of ≥ 20% at least four times during 4 weeks´ home monitoring (p between groups NS).

*Neonatal factors associated with spirometric values in children*

Multiple regression analysis was used to investigate the extent to which the lung function parameters in these children were associated with neonatal factors (Table 8). To compensate for the degree of prematurity, gestational age at birth was used as a covariate. The duration of intubation and of surfactant supplementation explained 30 to 41% of the variability of FVC, $FEV_1$ and PEF. The impacts of surfactant supplementation and the duration of intubation were additive. The duration of intubation was associated with the duration of oxygen therapy and with the diagnosis of CLD: each of these three covariates explained 15 to 30% of the variability of FVC, $FEV_1$ or PEF (data not shown). Surfactant supplementation explained about 10% of the variation in FVC, $FEV_1$ and PEF. Surfactant supplementation was also associated with a high SP-A/DPC ratio and short duration of ≥40% inspired $O_2$ therapy. The two latter variables were also associated with lung function (data not shown). Of the neonatal variables studied, only tracheobronchial stenosis in the newborn was associated with low $FEF_{50}$ in the children. In the present cohort of children

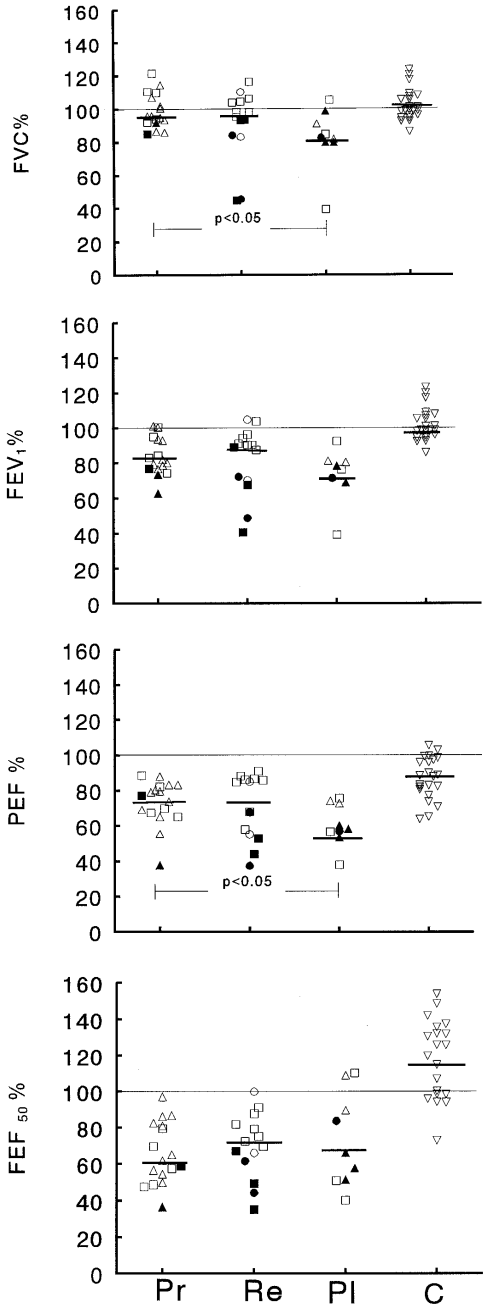


Figure 5. Study II. Spirometric values of the children measured in the clinic according to the outcome in the different study groups of surfactant therapy as percentages of the reference values. Medians are indicated by horizontal lines. Pr is prophylactic surfactant, Re rescue surfactant, and Pl the placebo group; C, control group (children born at term). Study 1 (○; ●, with CLD) was a randomized trial of surfactant therapy in RDS. Study 2 (Δ; ▲, with CLD) was a double-blind study of prophylactic surfactant. Study 3 (□; ■, with CLD) was a placebo-controlled comparison between prophylactic and rescue surfactant therapy. Significant differences between the placebo and the prophylactic surfactant groups are indicated in the figure. Significant differences were also observed ($p<0.05$) between full-term controls and the study groups except for FVC% between the controls and the prophylactic group. ∇, controls.

who were small preterm infants, the degree of prematurity had no detectable association with the lung function parameters. Neither the neonatal trial order nor the time of surfactant supplementation had any influence on the association between the neonatal variables and the spirometric values.

Table 8. Results of multiple regression analyses relating neonatal variables to lung function in childhood (subjects born before 30 weeks of gestation)* in the study II

| Lung function parameter | Covariates | Regression coefficient | SE | p values |
|---|---|---|---|---|
| FVC | Duration of intubation (d) | -0.24 | 0.062 | 0.004 |
| | Surfactant supplementation (1=yes, 0=no) | 13.14 | 4.98 | 0.012 |
| $FEV_1$ | Duration of intubation (d) | -0.23 | 0.062 | 0.0009 |
| | Surfactant supplementation | 9.60 | 4.62 | 0.045 |
| $FEF_{50}$ | Tracheo-bronchial stenosis (1=yes, 0=no) | -27.70 | 11.19 | 0.018 |
| PEF | Duration of intubation (d) | -0.20 | 0.064 | 0.004 |
| | Surfactant supplementation | 11.06 | 5.17 | 0.039 |

*Gestational age at birth was always included as a covariate; however, it was not significantly associated with the lung function parameters.

### 5.3. Peripheral blood lymphocyte subpopulations in schoolchildren born very preterm (Study III)

The 34 schoolchildren born very preterm and the 14 controls did not differ in age, height or weight. In seven children (three preterm-born children and four control children) home spirometry recording was not acceptable because of technical problems with the device (four children) or non-compliance (three children). For the 34 preterm-born children screened, the median (range) spirometric values in the clinic, expressed as percentages of the predicted values, were: FVC 96 (39-121)%, $FEV_1$ 84 (39-105)%, PEF 74 (38-91)% and $FEF_{50}$ 70 (35-110)%. In the control group, the corresponding median (range) spirometric values were: FVC 100 (84-124)%, $FEV_1$ 94 (79-118)%, PEF 86 (73-111)% and $FEF_{50}$ 106 (75-145)% of the

predicted values. The schoolchildren born very preterm had significantly lower $FEV_1$, PEF and $FEF_{50}$ values than the control group ($p < 0.05$). The median (range) of the number of positive bronchodilator tests at home was 3 (0-8) in the preterm-born children and 0 (0-2) in the control children ($p = 0.0006$). The median number (range) of abnormal PEF variations at home was 1 (0-9) in the preterm-born children and 0 (0-1) in the control children ($p = 0.006$).

*Peripheral blood lymphocyte subpopulations*

Flow cytometric measurements were obtained for all the control children and for 29 of the 34 children born preterm. Of these 29 preterm-born children, 14 had bronchial obstruction without an earlier diagnosis of asthma. The percentages of peripheral blood CD4+ and CD8+ T lymphocytes expressing the activation marker CD25 were determined in 20 children born very preterm. The percentage of CD4+ T lymphocytes and CD4/CD8 ratio were both significantly lower in the children born very preterm, and the percentage of CD16+CD56 cells (NK cells) were significantly higher than in the healthy controls ($p < 0.05$). Otherwise, the percentages of lymphocyte subsets in the two groups were comparable (Table 9).

In all the children, significant negative associations were observed between the number of ≥ 15% increments in PEF values after $\beta_2$ agonist during home monitoring and the percentage of CD4+ T lymphocytes ($r_s = -0.45$, $p = 0.008$) and the CD4/CD8 ratio ($r_s = -0.50$, $p = 0.003$) (Figure 6). In the very preterm-born schoolchildren with bronchial obstruction, the number of ≥ 15% increments in PEF values after the $\beta_2$ agonist had a significant negative association with the percentage of CD4+ T cells expressing CD 25 ($r = -0.75$, $p = 0.03$) (Figure 6). In all children, the number of ≥ 15% increments in PEF values after the $\beta_2$ agonist and the percentage of CD19+ lymphocytes expressing CD 23 tended to be associated ($r = 0.30$, $p = 0.08$).

*Serum markers of inflammation*

The number of blood eosinophils and the ECP and MPO values were measured in all the children. Serum ECP values were significantly higher in the preterm-born schoolchildren than in the healthy controls ($p = 0.03$). The number of blood eosinophils and the MPO values were similar in the two groups (Table 10). Parental smoking and ECP values were not significantly associated.

Table 9. Peripheral blood lymphocyte subsets in schoolchildren born very preterm and controls in the study III

| Cell surface antigen | Preterm-born children N = 34 | Healthy controls N = 14 | p values |
|---|---|---|---|
| CD3 | 67.9 (54.8-78.9) | 71.3 (60.9-78.2) | 0.15 |
| CD4 | 38.4 (25.7-52.5) | 42.2 (35.8-53.1) | 0.03 |
| CD8 | 28.8 (18.7-37.6) | 25.3 (17.5-36.3) | 0.18 |
| CD4/CD8 ratio | 1.4 (0.8-2.2) | 1.8 (1.0-2.6) | 0.03 |
| CD19 | 11.8 (5.2-25.8) | 9.8 (6.9-17.0) | 0.18 |
| CD23 | 10.6 (4.3-20.4) | 7.7 (2.6-22.4) | 0.66 |
| CD19/CD23 | 9.3 (3.6-20.3) | 5.8 (2.4-12.4) | 0.06 |
| CD16+CD56 | 11.5 (3.8-21.5) | 5.5 (4.0-18.0) | 0.03 |
| CD4/CD25 | 8.5 (5-14) | 9.1 (7.5-11.2)* | |
| CD8/CD25 | 3 (0-15) | 2.4 (1.9-2.7)* | |

Values of lymphocyte subsets are expressed as percentages of lymphocytes.
Values are expressed as medians (range).
*The reference values for CD4/CD25 and CD8/CD25 are taken from the reference Gemou-Engesaeth et al 1994.

Table 10. Numbers of blood eosinophils and S-ECP and S-MPO levels in schoolchildren born very preterm and controls in the study III

| | Preterm-born children N = 34 | Healthy controls N = 14 | p values |
|---|---|---|---|
| Blood eosinophils,109/l | 0.25 (0.05-0.96) | 0.15 (0.03-0.65) | 0.3 |
| Serum ECP, µg/l | 6.1 (1.8-50.6) | 2.9 (1.8-10.2) | 0.03 |
| Serum MPO, µg/l | 238 (43-1065) | 168 (127-320) | 0.3 |

Values are expressed as medians (range).
ECP=eosinophil cationic protein, MPO=myeloperoxidase.

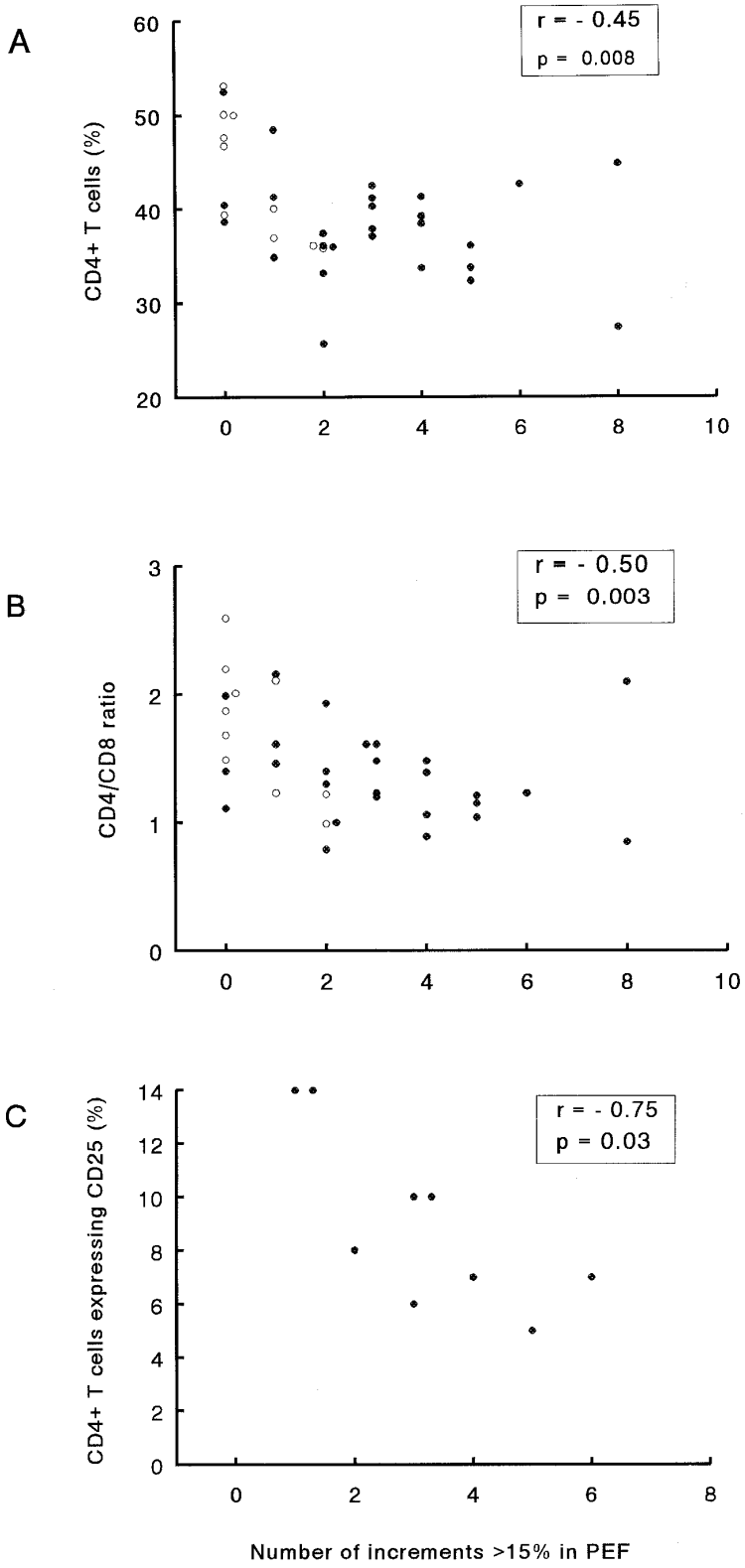


Figure 6. Study III. Relationship between the number of increments of ≥ 15% after terbutaline during home monitoring and (A) the percentage of CD4+ T cells, (B) the CD4/CD8 ratio in 26 very preterm-born children (●) and in 10 healthy controls born at term (○) and (C) the percentage of CD4+ T cells expressing CD25 in 9 very preterm-born children with bronchial obstruction (●).

## 5.4. The effect of inhaled budesonide on basic lung function in schoolchildren born very preterm (Study IV)

*Patient selection for budesonide treatment*

Of the 63 children screened, 21 (33%) met our criteria for a budesonide treatment trial and 18 completed the study. At entry, the median (range) spirometric values in the clinic were FVC 87 (67-121)%, $FEV_1$ 74 (51-95)%, PEF 65 (44-77)%, and $FEF_{50}$ 59 (35-103)% of the values of predicted for these 18 children. The mean (SD) age and height of the 18 children studied were 10.1 (1.8) y and -0.3 (1.6) SD.

*Spirometry in the clinic*

There were no statistically significant differences between the spirometric values at entry and after placebo treatment. The spirometric values before and after each treatment periods did not change during the 4 months treatment with budesonide; median $FEV_1$ 74% (51%-95%) to 74% (59%-92%), FVC 87% (67%-121%) to 90% (66%-120%) and $FEF_{50}$ 59% (35%-103%) to 55% (29%-104%). The median PEF values in the clinic tended after budesonide treatment to increase from 65% (44%-77%) to 66% (51%-91%) ($p=0.07$) (Table 11). The sum of the symptom scores decreased significantly during treatment with budesonide ($p<0.05$) (Table 12).

Table 11. Spirometry in the clinic in the study IV

| Parameter | Baseline | After placebo | After 1 mo budesonide | After 4 mo budesonide | p values |
|---|---|---|---|---|---|
| FVC | 87 (67-121) | 86 (64-131) | 88 (66-131) | 90 (66-120) | p=0.3 |
| $FEV_1$ | 74 (51-95) | 73 (55-96) | 75 (55-99) | 74 (59-92) | p=0.5 |
| PEF | 65 (44-77) | 61 (48-86) | 68 (52-92) | 66 (51-91) | p=0.07 |
| $FEF_{50}$ | 59 (35-103) | 53 (35-94) | 58 (42-89) | 55 (29-104) | p=0.7 |

Values are expressed as medians (range).
Lung function tests are expressed as percentages of the predicted values.

*Spirometry at home and respiratory symptoms*

A diurnal PEF variation of ≥ 20% was found at least four times during the 4 weeks´ home

monitoring in 10 of the study children (48%); the median (range) frequencies of diurnal PEF variation ≥ 20% were 3 (0-9) during the whole 4 weeks period, 2 (0-6) during the 2 weeks terbutaline treatment and 1.5 (0-5) during the placebo treatment. None of the control children had abnormal diurnal PEF variation, the median (range) frequencies of a diurnal PEF variation ≥ 20% in the control children being 0 (0-1) during 4 weeks home monitoring ($p < 0.01$). The long-term effects of terbutaline and placebo at home were evaluated by studying the mean morning and evening FVC, $FEV_1$ and PEF values. There were no significant differences in these parameters recorded at home during the 2 weeks terbutaline or placebo treatments. Neither did the PEF variation differ between these two periods. The median (range) frequencies of diurnal PEF variation ≥ 20% during the whole 4 months study period decreased significantly, from 1.5 (0-5) during the 2 weeks placebo treatment period to 0 (0-4) during last 2 weeks of budesonide treatment ($p<0.05$). During the 4 months treatment with budesonide, the median morning PEF level increased from 58% to 67% of that predicted ($p<0.01$). Correspondingly, the median morning $FEV_1$ level increased from 65% to 70% of that predicted ($p<0.05$). In the median evening PEF and $FEV_1$ and in the median morning FVC, the increase was significant only after treatment with budesonide for 1 month (<0.05 for all). During treatment with budesonide for 4 months, the median evening FVC values at home did not change (Table 12).

Table 12. Spirometry at home and respiratory symptoms in the study IV

| Parameter | After terbutaline (1) | After placebo (2) | After 1 mo budesonide (3) | After 4 mo budesonide (4) | p values |
|---|---|---|---|---|---|
| Symptom score | 26 (0-106) | 18 (0-119) | 3 (0-86) | 4 (0-104) | 2 vs 3 p=0.02 |
| PEF variation | 2 (0-6) | 1.5 (0-5) | 0.5 (0-3) | 0 (0-4) 2 vs | 3 p=0.05 |
| | | | | | 2 vs 4 p=0.02 |
| Morning PEF | 56 (28-94) | 58 (36-95) | 61 (36-100) | 67 (43-94) | 2 vs 3 p=0.008 |
| | | | | | 2 vs 4 p=0.005 |
| Evening PEF | 59 (38-92) | 59 (42-100) | 66 (40-92) | 67 (44-99) | 2 vs 3 p=0.04 |
| Morning $FEV_1$ | 67 (49-83) | 65 (46-90) | 69 (44-95) | 70 (48-89) | 2 vs 3 p=0.01 |
| | | | | | 2 vs 4 p=0.02 |
| Evening $FEV_1$ | 66 (50-85) | 66 (49-91) | 71 (53-96) | 71 (57-86) | 2 vs 3 p=0.02 |

Values are expressed as medians (range).
Lung function tests are expressed as percentages of the predicted values.

*Correlations*

Gestational age at birth correlated positively with the PEF increment after budesonide treatment in the clinic ($r_s$=0.54, p<0.05). Atopy was positively correlated with the increment in mean PEF value at home ($r_s$=0.48, p<0.05) but there was no correlation with smoking. The diurnal variation in PEF recorded during the treatment period with placebo and the short-term reversibility in response to terbutaline correlated significantly with the decrease in diurnal PEF variation during budesonide treatment ($r_s$ = 0.90 and 0.53, respectively, p < 0.05 for both)

## 5.5. Reproducibility of home spirometry in children with newly diagnosed asthma (Study V)

The characteristics of the 110 children included in this study are presented in Table 13. According to these lung function data, the study group comprised children with mild asthma. The mean duration of home spirometry recordings was 24 days (Table 13). During this period, the mean (SD) compliance in the home spirometry was 94% (7), range 63-100. The mean (SD) difference between the calibration of the home spirometry before and after home monitoring of spirometry was 1.7% (1.5), range 0-8%.

Table 13. Characteristics of the asthmatic children studied (N = 110) in the study V

| | | | Mean | SD | Range |
|---|---|---|---|---|---|
| Age, yr | | | 7.4 | 1.6 | 5.0-10.9 |
| Height, cm | | | 126.1 | 11.2 | 104.6-157.1 |
| Weight, kg | | | 27.5 | 8.0 | 16.3-52.2 |
| Sex | F | 42 (38%) | | | |
| | M | 68 (62%) | | | |
| Duration of home recordings, d | | | 23.6 | 7.2 | 7-43 |
| $FEV_1$ (% of pred) in the clinic | | | 91.8 | 11.4 | 58.8-116.9 |
| FVC (% of pred) in the clinic | | | 98.7 | 11.8 | 64.8-141.1 |
| PEF (% of pred) in the clinic | | | 83.7 | 13.4 | 58.1-130.5 |

pred = predicted

The reproducibility of the home measurements was estimated by calculating the percentage of home spirometry measurements that met levels of reproducibility ≤ 5% (ATS criteria), <10% and <15%. Children were assigned to three categories according to age (5-6 years, 7-8 years and 9-10 years), and to height (< 121 cm, 121-135 cm and 136-150 cm).

The mean (SD) percentage of reproducible spirometry measurements, according to the ATS criterion was 76.5% (16.6), range 21-100% in the whole study population. The percentage of reproducible home measurements was significantly lower at 5-6 years than at 9-10 years (p=0.007) (Table 14). In the < 121 cm height group the percentage of reproducible spirometry values was significantly lower than in the 136-150 cm height group (p=0.002), and in the 121-135 cm group lower than in the 136-150 cm group (p=0.03) (Table 14).

Table 14. Age and height in relation to the percentage of reproducible home measurements according to the criterion of ATS in the study V

| | | N | Mean | SD | Anova p | Pairwise comparisons | p values |
|---|---|---|---|---|---|---|---|
| Age, yr | 5-6 | 51 | 72.8 | 18.6 | 0.02 | 5-6 vs 7-8 | 0.22 |
| | 7-8 | 38 | 77.1 | 13.8 | | 5-6 vs 9-10 | 0.007 |
| | 9-10 | 21 | 84.5 | 13.7 | | 7-8 vs 9-10 | 0.10 |
| Height, cm | <120 | 39 | 71.6 | 19.2 | 0.007 | <121 vs 121-135 | 0.15 |
| | 121-135 | 50 | 76.6 | 14.1 | | <121 vs 136-150 | 0.002 |
| | 136-150 | 21 | 85.6 | 13.4 | | 121-135 vs 136-150 | 0.03 |
| All | | 110 | 76.5 | 16.6 | | | |

As expected, height was strongly correlated with age in the children (r=0.89, p<0.0001). In regression analysis, age and height were included separately, and adding the two did not significantly improve the prediction of the dependent variable. Both age and height showed significant linear relations to the percentages of reproducible home spirometry measurements. The regression lines estimated were: percentage of reproducible home measurements = 55.6 + 2.82 x age (year) and = 22.6 + 0.43 x height (cm). Baseline spirometry (PEF, $FEV_1$ and FVC) as litres correlated significantly with the percentages of reproducible home measurements (r =

0.34, r=0.34 and r=0.37, respectively, $p<0.001$ for all). However, no correlation was found when the spirometry values were expressed as percentages of the values predicted according to the level of bronchial obstruction.

At the <10% level of reproducibility, the mean (SD) percentage of reproducible spirometric measurements for the whole study population was 89% (12). In the 5- to 6-year age group (n= 51) the mean (SD), expressed as a percentage of the reproducible spirometry values, was 86.2% (14.1), in the 7- to 8-year group (n=38) 89.3% (9.1) and in the 9- to 10-year group (n=21) 93.2% (10.4). The percentage of reproducible measurements in home spirometry was significantly lower in the 5- to 6-year age group than in the 9- to 10-year age group (p=0.02). Correspondingly, in the < 121 cm height group the percentage of reproducible spirometry values was significantly lower than in the 136-150 cm height group (p=0.006) (Figure 7).

Using the level of reproducibility <15%, the mean (SD) percentage of reproducible spirometry measurements was 95% (9) for the whole study population. In the 5- to 6-year age group (n= 51) the mean (SD) percentage of reproducible spirometry values was 93.2% (10.8), in the 7- to 8-year group (n=38) 95.5% (4.5) and in the 9- to 10-year group (n=21) 95.7% (8.8). The differences between the age groups and height groups were no longer statistically significant.

The correlations between age and height versus the percentage of reproducible home measurements were: r=0.28, p=0.004 and r=0.29, p=0.002 (ATS criteria), r=0.24, p=0.01 and r=0.26, p= 0.007 (at the <10% level of reproducibility) and r=0.15, p=0.12 and r=0.18, p=0.07 (at the <15% level of reproducibility) (Figure 7).

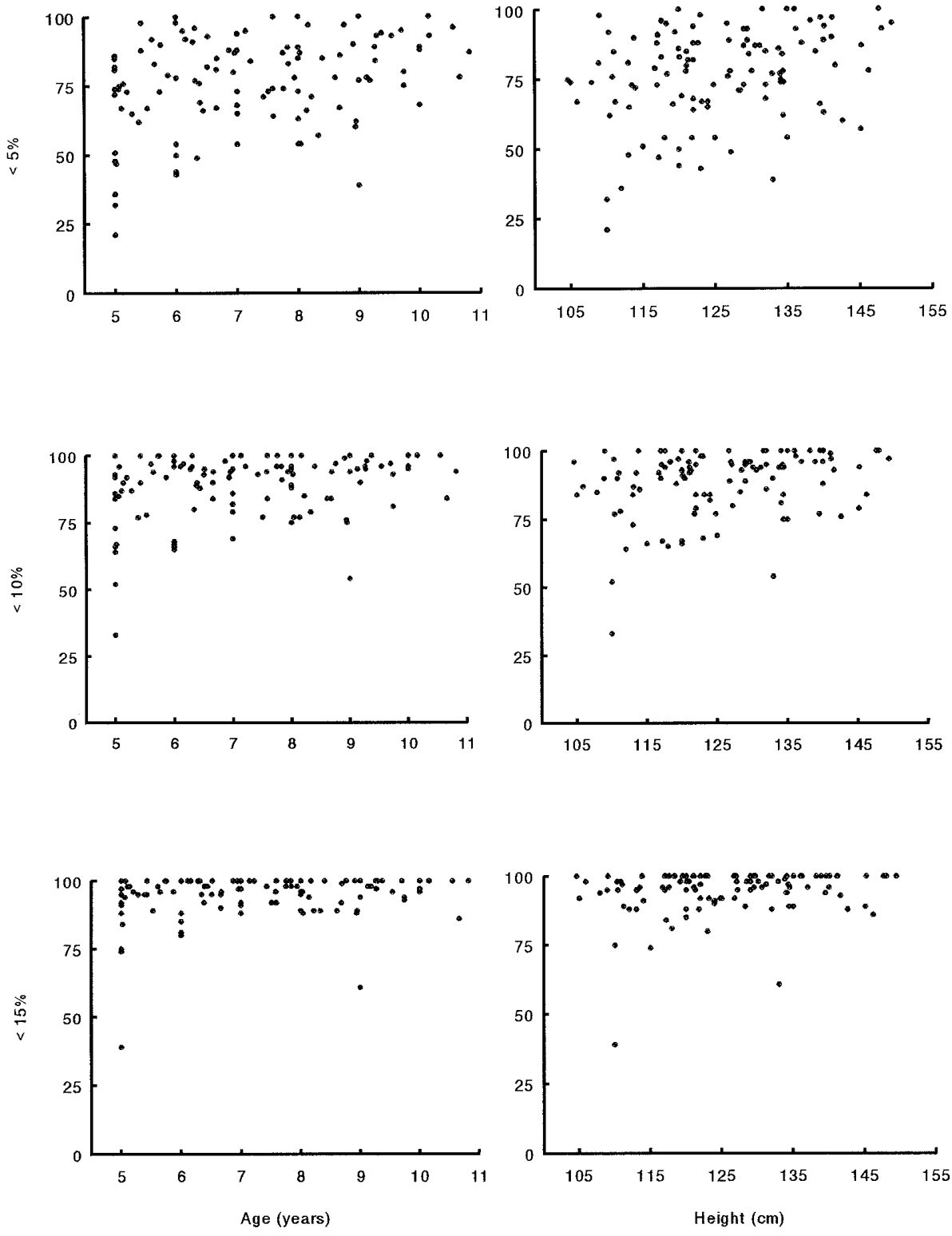


Figure 7. Reproducible spirometry measurements at home: the level of reproducibility ≤5% (the criterion of the American Thoracic Society), <10% and <15% as percentages of all registered spirometric tests in all 110 children with newly diagnosed asthma related to the age and height of the children.

# 6. DISCUSSION

It is of concern to many that prematurity itself and CLD in particular lead to "second-class" lungs in later childhood and adult life (Wohl 1990). For example, in our cohort of 63 schoolchildren born very preterm, respiratory symptoms, bronchial obstruction, bronchial lability and increased bronchial responsiveness were common, regardless of CLD. More than half the children with bronchial obstruction responded to a $\beta_2$ agonist or had abnormal diurnal PEF variation. The variability of the bronchial obstruction could be detected only by recording PEF values at home for a prolonged period. Our cohort had significantly lower $CD4^+$ T-cell percentages and CD4/CD8 ratios than healthy controls. A relationship between bronchial lability and imbalance of T-cell subpopulations was observed. Our results provide suggestive evidence that bronchial lability in schoolchildren born very preterm has an immunological basis. Inhaling of budesonide for 4 months had no significant effect on basic lung function in our cohort. A significant but small decrease in bronchial lability and respiratory symptoms was observed during this treatment. We also present evidence that the beneficial pulmonary effects of neonatal therapy with natural surfactant extend into later childhood.

## 6.1. Methodological discussion

In the clinic, we used conventional flow volume spirometry, the bronchodilator test, the dosimetric histamine challenge test and conventional methods to evaluate inflammatory changes. At home, the children recorded lung function with a novel device, a computerized home spirometer. Measuring pulmonary function throughout successive days provides information that might be missed with the single "snapshot" obtained in the office. Home monitoring is therefore an important way to measure changes in pulmonary function, both within a day and from day to day. The presence of bronchial lability is reflected by reduced PEF values during the early morning hours. Accordingly, a response to a $\beta_2$ agonist may be observed more frequently during home spirometry. Values of PEF tend to be lowest on waking, and to reach maximal values between noon and early evening (Enright et al 1994). During homemonitoring it is recommended to make measurements on rising and early evening, and in addition one other measurement in the

early afternoon (Quanjer et al 1997). In our study measurements with the spirometer was recorded only every morning and evening because of practical aspects.

Many variables may affect the validity of lung function testing in children at home. Of these variables, the reliability of lung function measurements, including compliance and the reproducibility of the measurements, are all of crucial importance. Data on the reproducibility of individual measurements are especially sparse, and we do not know at what age children can perform spirometry reliably at home. It is known that absolute values of PEF obtained with standard devices, such as mini-flow meters, may be inaccurate, especially in children (Sly et al 1997). Conventional recordings of PEF in asthma performed with hand-held mini PEF meters, as compared with devices with electronic storage capabilities, may overestimate the number of recordings, containing a large number of retrospective and even invented data (Chowienczyk et al 1994, Quirce et al 1995, Redline et al 1996, Verschelden et al 1996). Some studies comparing simple PEF meters and devices with electronic storage capabilities have shown that compliance decreased after 2 weeks of home monitoring (Redline et al 1996, Verschelden et al 1996). Our methodological study of the reproducibility of home spirometry in children suggests that the device used in our study is suitable for clinical studies. Most of the 110 children with newly diagnosed mild asthma aged from 5 to 10 years could perform reproducible spirometry tests during home monitoring, although there was wide individual variation. This ability is related to the age and height of the child. However, a considerable proportion of the tests do not meet the criteria of reproducibility. In order to improve the reproducibility of home spirometry and study results, the non-reproducible measurements should be excluded from the analysis. The overall compliance in home spirometry during the 3 weeks of recording for the present asthma study was excellent; 94% of the required tests were performed. This new computerized home spirometer affords the following advantages: (a) compliance control of spirometry and medication by recording the time of measurement and inhalation of the drug, (b) control of the reproducibility and acceptability of measurements and (c) the possibility to calibrate the device. In the present cohort of 63 schoolchildren born very preterm, 89% of the measurements during home monitoring were acceptable according to the ATS criteria.

### 6.2. Bronchial obstruction

The finding of bronchial obstruction in our CLD children agrees with earlier observations (Smyth et al 1981, Bader et al 1987, Andreasson et al 1989, Northway et al 1990, Kleine et al 1990, Hakulinen et al 1990, Blayney et al 1991, Parat et al 1995, Hakulinen et al 1996, Koumbourlis et al 1996, Jacob et al 1997, Jacob et al 1998, Gross et al 1998). In our children, the level of obstruction was as remarkable as in the study of Jacob et al (1998), in which the children selected resembled our cohort and were as premature as ours. As the numbers of very preterm born survivors have increased, mild to moderate obstructive airway dysfunction at school age has also been found in children without a history of CLD, as in our study (Bertrand et al 1985, Galdes-Sebaldt et al 1989, Chan et al 1989c, Lebourges et al 1990, Kleine et al 1990, Hakulinen et al 1996, Jacob et al 1997). The definition of CLD by Shennan et al (1988), also used in the present study, seems to be a sensitive predictor of abnormal pulmonary outcome in schoolchildren born very preterm; 83% of the CLD children had bronchial obstruction. However, bronchial obstruction was also observed in 31% of the non-CLD children.

We found that the degree of bronchial obstruction was associated with the duration of mechanical ventilation and the duration of oxygen therapy. This suggests that the severity of neonatal disease and/or the duration of the neonatal treatment influences pulmonary function at school age. Prematurity alone was also a risk factor for abnormal pulmonary function in our schoolchildren born very preterm. In agreement with earlier studies, our schoolchildren born preterm without respiratory disease (RDS or CLD) had reduced $FEV_1$, PEF and $FEF_{50-75}$ values in comparison with healthy controls (Figure 2) (Coates et al 1977, Bertrand et al 1985, Mansell et al 1987, Galdes-Sebaldt et al 1989, Parat et al 1995). In contrast to previous studies (Bader et al 1987, Mansell et al 1987, Galdes-Sebaldt et al 1989, Chan et al 1989c), birth weight and gestational age were not associated with pulmonary function, obviously because of the narrow range of gestational ages in our children. In our study, reduced fetal growth had no effect on lung function at school age. This study was not ideal for investigating such a hypothesis, because the IUGR group was small, comprising only 11% of the children.

In agreement with previous studies (McLeod 1996, Gross et al 1998), FVC was significantly smaller in our CLD group than in the non-CLD and control groups. Because whole-body plethysmographic measurements were not performed in the present series, it is not certain whether these lower FVC values reflect smaller thoracic gas volumes or air trapping because of the bronchial obstruction.

### 6.3. Bronchial responsiveness and lability

In contrast to previous studies, we found that only one of our children showed a significant response to a $\beta_2$ agonist in the conventional bronchodilator test in the clinic. A weak response ($\geq$ 10%) was noted in 8% of our children, all but one in the CLD group. In a previous study by Northway et al (1990), responsiveness of $\geq$ 10% in the bronchodilator test was observed in 44% of the patients with a history of CLD as compared with our 22%. Andreasson et al (1989) and Kleine et al (1990) found that in preterm born and ventilated children of school age, after $\beta_2$ agonist inhalation, $\Delta FEV_1$ was higher than in the reference group, regardless of CLD. Bronchodilator responsiveness was observed twice as often in preterm children with previous CLD (47%) as in preterm children without CLD (25%) or in the term control group (21%) (Gross et al 1998). However, in our study during home monitoring, the bronchodilator test was positive in 39% in CLD group and also in 40% of the non-CLD group. In addition, 16% of the CLD and 24% of the non-CLD group had increased diurnal PEF variation, indicating bronchial lability. A significant response in the bronchodilator test and/or abnormal diurnal PEF variation, bronchial lability, were observed in 48% of all children and in 66% of the 29 children with verified obstruction. None of our control children had abnormal diurnal PEF variation or a positive bronchodilator test during home monitoring. In our study significantly more schoolchildren born preterm without a history of RDS and CLD had bronchial lability than the healthy controls The explanation for discrepancy between the results for bronchial responsiveness at home and in the clinic might be differences in the study population and the fact that a response to a $\beta_2$ agonist may be observed more frequently during home monitoring.

We found that CLD children were significantly more responsive to histamine than non-CLD children and healthy controls. In our study the primary explaining factor for bronchial responsiveness was the duration of ventilator treatment. At school age, bronchial hyperresponsiveness has been observed not only in children with CLD (Smyth et al 1981, Northway et al 1990), but also in prematurely born children without CLD (Bertrand et al 1985, MacLuscy et al 1986, Galdes-Sebaldt et al 1989). In contrast to previous reports (Northway et al 1990), we obtained data on respiratory symptoms from the majority of the children with verified obstruction. In our study, there tended to be an association between respiratory symptoms and $PD_{15}FEV_1$ values. This suggests that bronchial responsiveness is of clinical significance. Those children whose $PD_{15}FEV_1$ was < 1 mg were all symptomatic. Similarly, Chan et al (1989c) reported a strong relationship between respiratory symptoms and airway responsiveness.

### 6.4. Pathogenesis leading to lung function abnormalities

In asthma, bronchial hyperresponsiveness is associated with inflammation of the bronchial mucosa. In children with CLD, however, this association has not been verified. In a pilot study, we treated 18 children with bronchial obstruction, increased responsiveness to a $beta_2$ agonist, and/or increased diurnal variation in PEF without earlier diagnosed asthma for 4 months with inhaled GC. In contrast to asthma, basic lung function in our selected group of very preterm born schoolchildren was not significantly affected by budesonide treatment for 4 months, although both respiratory symptoms and diurnal PEF variation decreased during the treatment. This finding suggests that lung function abnormalities in schoolchildren born very preterm may be due to structural changes resulting from the early lung tissue injury and the abnormal pattern of remodelling. Increased bronchial responsiveness and bronchial lability may be aggravated by structural airway narrowing and may be a reflection of altered airway calibre or airway wall thickness (Chan et al 1989a, Northway et al 1990). In our study, spirometric values in the clinic showed a significant correlation with bronchial responsiveness and bronchial lability. Airway obstruction in schoolchildren born very preterm may be related to submucosal fibrosis, or hypertrophy, or hyperplasia of bronchial smooth

muscles (Margraft et al 1991, Chan and Silverman 1993).

The persistent pulmonary dysfunction in children born very preterm may in part be due to an immunological mechanism. In our study, as compared with the healthy controls, the schoolchildren born very preterm with lung immaturity had a significantly lower $CD4^+$ T-cell percentage and CD4/CD8 ratio. In addition, the NK-cell percentage and the S-ECP values were significantly higher. A weak but significant negative association was observed between the bronchial responsiveness in PEF after a $\beta_2$ agonist during home monitoring and the $CD4^+$ T-cell percentage and the CD4/CD8 ratio, suggesting a relation between bronchial lability and imbalance of T-cell subpopulations. Our findings suggest that bronchial lability is associated with alterations in the balance of T-cell subsets and possibly with abnormal $CD4^+$ T-cell function.

It is known that $CD4^+$ T lymphocytes are important regulators in the human immune system. A low CD4/CD8 ratio is a hallmark of intense, chronic immune responses, such as allograft rejection, graft-versus-host disease, and haemophilia (Amadori et al 1995). In asthma, the CD4/CD8 ratio is constant and does not differ from that of non-asthmatic groups (Corrigan et al 1988). The cause of imbalance in the CD4/CD8 ratio among very preterm born schoolchildren is not known. It may be a consequence of lung injury during infancy. It could also be a factor maintaining lung function abnormalities during childhood. Recent observations by Amadori et al (1995) suggest that the CD4/CD8 ratio is genetically controlled. It is possible that, in infants born very preterm, the early inflammatory process leading to CLD and lung function abnormalities is more easily triggered in those individuals who are genetically susceptible to a low CD4/CD8 ratio. Originally, CLD was observed primarily in preterm infants with severe RDS who had been exposed to high oxygen concentrations and positive airway pressures. In recent years, however, a less severe form of CLD has frequently been observed in infants with mild initial RDS, if any. This change in the clinical pattern suggests that additional risk factors differing from those associated with the severity of acute neonatal respiratory disease are responsible for the development of lung function abnormalities in susceptible infants (Rojas et al 1995). In the present study, ECP, but not MPO, levels in peripheral blood were significantly higher in schoolchildren born very

preterm than in controls. An increased serum level of ECP is considered to be a sign of activation of eosinophils during an inflammatory process in the airways (Bousquet et al 1990). A possible explanation for

the increased levels of blood ECP is parental smoking. Serum ECP values in young children have been shown to be related to maternal smoking (Lodrup Carlsen et al 1998). However, in our study parental smoking and ECP values did not have a significant positive association.

The course of CLD is considered to have four overlapping stages representing early inflammation, its resolution by the age of 1 month, repair and remodeling of acute lung injury, largely completed at 1 year of age and finally lung growth and development (Kotecha and Silverman 1998). Our results, however, provide, evidence that immunological abnormality still persists at school age. According to previous intervention studies and the present pilot study, the potential efficacy of inhaled GC in the treatment of CLD at school age is modest. The development of new therapies will depend on the results of future studies of this possibly chronic immunological process.

### 6.5. Effect of surfactant treatment

Given the evidence that the long-term outcome at school age is related to prematurity itself, rather than to acute lung injury alone, the prospects for preventive intervention of CLD in the premature would appear to be restricted. However, we present evidence that the beneficial pulmonary effects of natural surfactant therapy at birth extend into later childhood. Our follow-up study of small preterm infants participating in randomized studies of exogenous natural surfactant supplementation demonstrates that human surfactant given prophylactically at birth or early in RDS significantly improves FVC and PEF values at school age. The interpretation of the results is complicated because of the relatively small sample size and of multiple comparison of the data. Because, among the treated infants, early deaths due to respiratory failure were fewer than among the placebo-treated children (Hallman et al 1985, Merritt et al 1986, Merritt et al 1991) the number of infants with severe lung function abnormalities lost to follow-up was greater in the placebo-treated group. Therefore the

present results are likely to underestimate the beneficial long-term effects of exogenous surfactant. Multiple regression analysis further suggested that the factor primarily responsible for the favourable outcome was exogenous surfactant. The improved pulmonary outcome of the surfactant recipients may have been

due to the immediate improvement in lung function, resulting in shorter exposure to high levels of inspired oxygen and to high ventilatory pressures. Additionally, surfactant may protect against development of airway epithelial lesions during artificial ventilation (Robertson et al 1992) and may ameliorate hyperoxic lung injury (Fracica et al 1994). Both barotrauma and hyperoxia cause significant lung and airway injury (Jackson et al 1990). In the present study, a shorter duration of oxygen treatment (>40%) was significantly associated both with surfactant supplementation at birth and with higher FVC and $FEV_1$ values at school age. The surfactant index, the SP-A/DPC ratio during the first day of life, which is known to be associated with survival without CLD (Hallman et al 1991), was additionally found to be significantly associated with surfactant therapy and with better lung function values at school age. However, regardless of surfactant therapy, bronchial obstruction associated with bronchial lability and increased bronchial responsiveness remain common in schoolchildren born very preterm. Neonatal variables affecting the pulmonary outcome at school age negatively were duration of intubation and of $O_2$ therapy. Therefore, it is possible that shortening and modifiying the assisted
ventilation and $O_2$ therapy by improved management practices or by preventive treatments would further improve the pulmonary outcome in small preterm infants.

### 6.6 Lung function abnormalities in asthma and in schoolchildren born very preterm: risk factors and immunological changes

The possible link between asthma-like lung function abnormalities and respiratory symptoms in preterm born schoolchildren and asthma is unclear. In asthma, the most characteristic lung function features are bronchial lability, including short-term changes in the airway calibre, reversible airflow limitation, BHR and eosinophilic and lymphocytic airway inflammation. CLD is defined as a chronic disorder characterized by limitation of airflow that is partially

reversible. It may be accompanied by BHR. The immunological changes, including the present finding of imbalance in the CD4/CD8 ratio in the peripheral blood are different from the findings reported in asthma. Atopy, the strongest identifiable factor predisposing to asthma, was not a risk factor in lung function abnormalities in schoolchildren born very preterm in the present study. On the ther hand, parental smoking associated significantly with flow values at school age, increasing the risk of bronchial obstruction. Therefore, parental smoking seems to predispose to lung function abnormalities of preterm children at school age.

Although asthma and CLD show some similarities, they are different disease entities (Figure 8). Cooccurrence of the two diseases is likely to occur and prematurity could be regarded as a risk factor for asthma. In our study, 28% of the children in the CLD group and 16% in the whole group of schoolchildren born very preterm had physician-diagnosed asthma. These figures are considerably higher than the current asthma prevalence of 4-6% in Finland (Koskela et al 1996).

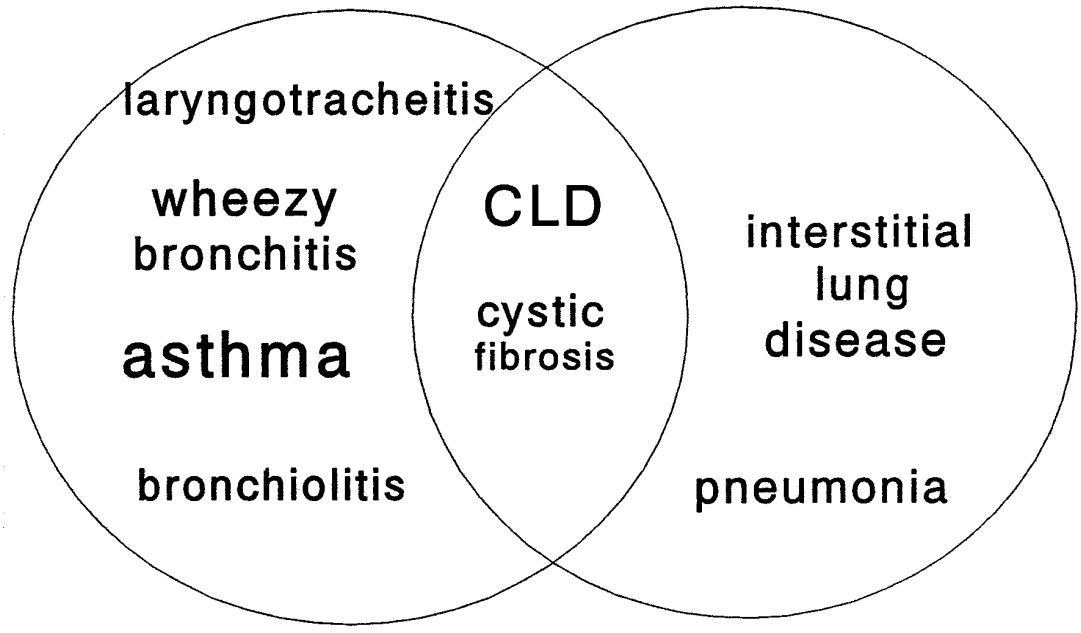


Figure 8. Simplified interrelationships between different lung disorders. Laryngotracheitis, wheezy bronchitis, asthma and bronchiolitis are bronchial diseases. Interstitial lung disease and pneumonia are lung parenchyma diseases. Chronic lung disease of children born very preterm, CLD and cystic fibrosis have characteristics of both main disease groups.

## 7. SUMMARY

We evaluated basic spirometry, bronchial lability and responsiveness in a total of 63 schoolchildren born very preterm. Mean birth weight was 1044 g, mean gestational age 27.6 weeks and mean age at study 10.0 years. Eighteen children had a history of CLD. Forty of these children, who were all born with an immature surfactant system before 30 weeks gestation, had participated during infancy in trials of human surfactant therapy at the Children´s Hospital, University of Helsinki, Finland. Thirty-four of these 40 children participated in the follow-up study investigating the associations of lymphocytes or serum inflammatory markers with obstructive lung disease and bronchial lability in schoolchildren born very preterm. Eighteen of the 63 children born very preterm, who were without a previous diagnosis of asthma but had bronchial obstruction and/or bronchial lability, were selected for the budesonide trial.

In a methodological study assessing the reproducibility of home monitoring in children with newly diagnosed asthma, 110 outpatients born at term, at a mean age of 7.4 years, were selected as our series for evaluating the reproducibility of home spirometry.

In all the 63 schoolchildren born very preterm, flow-volume spirometry, a bronchodilator test and home monitoring of PEF values twice daily for 4 weeks with and without a $\beta_2$ agonist were performed with a novel Data Storage Spirometer. The spirometric values in the office and the results of home monitoring were compared with those for a control group of children born at term (n=25). A histamine challenge test was performed in 29 schoolchildren born very preterm and in 14 healthy controls. Eighteen of these prematurely born schoolchildren were first treated with terbutaline inhalations for 4 weeks followed by inhaled placebo for the next 2 weeks, and then with inhaled budesonide for 4 months. Daily symptom scores, spirometric values in the clinic and at home at the beginning and end of each treatment period, were evaluated. We studied lymphocyte subsets in peripheral venous blood by flow cytometry in 29 children. Serum ECP and MPO levels, and the association between serum ECP and MPO levels, lymphocyte subsets, and lung function were also determined.

In order to evaluate the reproducibility of home spirometry in children with newly diagnosed asthma according to the criteria of the ATS, flow-volume spirometry was performed in 110 children in the clinic. Spirometric values were then monitored twice daily at home for 24 days (mean).

In the clinic spirometric values indicating bronchial obstruction were observed in 83% of children in the CLD group and in 31% of children in the non-CLD group. Of the children with obstruction, 62% reported respiratory symptoms during the past year. All spirometric parameters except $FEV_1$/FVC were significantly lower in the CLD group than in the non-CLD group. All spirometric values were significantly lower in the preterm group than in the full-term controls. Children without a history of RDS and CLD had significantly lower flow values than the healthy controls. A significant response in the bronchodilator test and/or abnormal diurnal PEF variation indicating bronchial lability were observed in 48% of the children tested. The CLD children were significantly more responsive to histamine than the non-CLD children and healthy controls. Bronchial obstruction was associated with the duration of mechanical ventilation and oxygen therapy. Parental smoking was a risk factor for bronchial obstruction at school age. PEF and FVC values were higher in those schoolchildren born very preterm who, during early infancy, had received surfactant compared with placebo. In multiple regression analysis, the independent variables associated with higher spirometric parameters were surfactant therapy and short intubation time after birth.

As compared with the healthy controls, the schoolchildren born very preterm had significantly lower $CD4^+$ T-cell percentages and CD4/CD8 ratios, whereas the NK-cell percentages and S-ECP values were significantly higher. These very preterm born schoolchildren had significantly lower spirometric values, except for FVC, than the control group. A weak, but significant, negative association was observed between the bronchial responsiveness in PEF after a $\beta_2$ agonist during home monitoring and the $CD4^+$ T-cell percentage and the CD4/CD8 ratio, indicating a relation between bronchial lability and imbalance of T-cell subpopulations. After treatment with budesonide for 4 months, spirometric values in the clinic were not significantly changed.

In our methodological study, the mean compliance in performing the spirometric tests at home was 94%. In this study of newly diagnosed asthma in children aged 5 to 10 years, born at term, the mean percentage of reproducible spirometric measurements was 77%, although there was wide individual variation (range 21-100%). The percentage of reproducible home measurements was significantly related to age and height of the children. We concluded that most children aged 5 to 10 years can perform reproducible spirometric tests during home monitoring, although there was wide individual variation. Younger children were less likely to perform reproducible tests than were older children; almost all the children aged 9 years or more, but also over half of the children aged 5-8 years, can perform reproducible spirometric tests at home.

## 8. CONCLUSIONS

1. Respiratory symptoms, bronchial obstruction, bronchial lability and increased bronchial responsiveness are common in schoolchildren born very preterm, regardless of CLD. Half the children with obstruction responded to a $\beta_2$ agonist or had abnormal diurnal PEF variation. The variability of the bronchial obstruction could be detected only by recording PEF values at home for a longish period. Bronchial obstruction was associated with the duration of mechanical ventilation and oxygen treatment. Parental smoking was a risk factor for bronchial obstruction at school age. A regular follow-up programme of lung function seems to be essential throughout childhood including PEF monitoring at home especially children with respiratory symptoms.

2. At school age, PEF and FVC values were significantly higher in those who received surfactant compared with placebo during infancy, and in multiple regression analysis, the independent variables associated with higher spirometric values were surfactant therapy and a short intubation period after birth. This serves an evidence that the beneficial pulmonary effects of natural surfactant therapy extend into later childhood. Our results suggest that active prevention and treatment of lung function abnormalities during the perinatal period prevent lung function abnormalities in children born very preterm. At school age, avoidance of tobacco smoke, management of symptoms and heath maintenance are important .

3. As compared with the healthy controls, the schoolchildren born very preterm with lung immaturity had a significantly lower percentage of $CD4^+$ T-cells and CD4/CD8 ratios, whereas the NK-cell percentage and the S-ECP values were significantly higher. A weak but significant negative association was observed between bronchial responsiveness in PEF after a $\beta_2$ agonist during home monitoring and the $CD4^+$ T-cell percentage and the CD4/CD8 ratio, suggesting a relationship between bronchial lability and imbalance of T-cell subpopulations. Our results provide suggestive evidence for an immunological basis of bronchial lability in schoolchildren born very preterm. The immunological changes, including the present finding of imbalance in the CD4/CD8 ratio in the peripheral blood, are different from those reported in asthma.

Inhaled GC treatment for 4 months had no significant effect on basic lung function in our selected group of very preterm born schoolchildren, although both respiratory symptoms and diurnal PEF variation tended to decrease during the treatment.

4. Most of the children with newly diagnosed mild asthma aged from 5 to 10 years can perform reproducible spirometry tests during home monitoring, although there was wide individual variation. The reproducibility improved with the age of the child. However, a proportion of the tests did not meet the criteria of reproducibility. In order to improve the reproducibility of home monitoring, the non-reproducible measurements should be excluded from the analysis.

## 9. ACKNOWLEDGMENTS

This study was carried out at the Allergy Unit of Skin and Allergy Hospital, University of Helsinki, between 1994 and 1998. Study children treated at the neonatal intensive care unit of the Hospital For Children and Adolescents, University of Helsinki, were examined at school age at the Allergy Unit of Skin and Allergy Hospital.

I am greatly indebted to Professor Jaakko Perheentupa, MD, Head of the Children´s Hospital and Professor Kari Raivio, MD, Professor of Perinatology, University of Helsinki, for granting me permission to study children born very preterm. I wish to express my sincere gratitude to Professor Annamari Ranki, MD, Head of the Skin and Allergy Hospital, for giving me the opportunity to do this study at Skin and Allergy Hospital. I express my sincere gratitude to Docent Tari Haahtela, MD, Head of the Allergy Unit of Skin and Allergy Hospital, for placing the excellent research facilities of the hospital at my disposal. His support has been extremely valuable.

I would like to express my sincere thanks to Marja Tevaluoto-Aarnio, MD, who arouse my interest to study the lung function of children born very preterm and introduced me to the fascinating field of pediatric pulmonology.

My deepest gratitude I owe to Docent Markku Turpeinen, MD, my principal supervisor for his excellent advice and support during this eventful work. His experience in the field of pediatric pulmonology has been fundamentally important for this work`s succesful completion. He also guided me into the world of scientific work. I have learned from him a lot about the importance of a critical mind in scientific work. His patient supervision, interest, and never-failing support made this study come true.

I also wish to express my special thanks to my second supervisor Professor Mikko Hallman, Head of the Department od Pediatrics, University of Oulu, for his excellent advice and support during my work. His experience in the field of science and profound knowledge on neonatal pulmonary diseases has been very important for this work´s succesful completion. His optimistic attitude, help, and support, despite several duties, has been invaluable.

I would like to express my sincere thanks to Docent Kirsti Heinonen, MD and Docent Päivi Piirilä, MD, for their most valuable criticism and advice during the preparation of the final manuscript of this thesis.

I cordially thank all my collaborators. I owe thanks to Arja Hakulinen, MD, for help, encouragement and cheerful company during these years and her participation in these trials. I sincerly thank Hanna Raitio, MD, and Kurt Nikander, BA, for their participation in these trials. Hanna Raitio, MD, Kirsi Järvinen, MD, and Pamela Österlund, BA, are also acknowledged for their friendly advice and practical assistance in practicals of flowcytometry. I also thank Docent Kaisu Juntunen-Backman, MD and other colleagues and friends at the

Allergy Unit of Skin and Allergy Hospital for interest and sympathy.

I owe many thanks to nursing staff of the study, Tuula Koljonen, Leena Ingelin-Kuortti and Ilona Kuistio for skillfull assistance throughout the course of the study. Warm thanks are due to many colleagues and nurses at the Allergy Unit of Skin and Allergy Hospital, University of Helsinki and other participating hospitals for their assistance and positive attitude during these years.

I am very grateful to Tuija Poussa, BA, for her skilful and friendly help and advice in statistical problems. I also want to thank Jean Margaret Perttunen, for the language revision of the final manuscript of this thesis.

All parents and children participarting in the study are warmly acknowledged. Without their positive attitude towards research, this study could never have been accomplished. Special thanks are also due to healthy subjects who participated in this study as volunteers.

My very warmest thanks belong to my family, to Markku and our lovely children Aino, Jaakko, Tapani, Oili-Maija, Ruut-Maaria and Anni-Kaisa. I thank for my husband and our children for all the love, understanding and support I have received from them during the years of the study. You all are the best ones in my life. Finally, I also want to thank my mother and father for care and love during my whole life.

This work was financially supported by grants from the Foundation for Allergic Research, the Finnish Medical Society Duodecim, The Finnish Society of Allergology and Immunology, the Foundation of Helsinki University Central Hospital, the Orion Corporation Research Foundation and AstraZeneca R&D, Lund, Sweden.

Espoo, December 1999

Anna Pelkonen

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