Lung function in school children born early. Identifying those at future risk.

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Work undertaken during thesis

Study set-up

- Participated in ethics application
- Developed consent and assent forms for study
- Developed all paperwork related to home visits, including adapted ISSAC surveys, invite letters, GP letters, clinical research forms and the data collection pro-forma
- Developed Standard Operating Procedures (SOP's) and associated paperwork
    - PGD, lone worker and inappropriate contact of family of deceased child
- Set-up testing protocols for home visits
- Set-up and maintained recruitment databases
    - Formulated database of all potential recruits for RHiNO study
    - Selected potential recruits from prior RANOPS cohort by geographical area and age
    - Regularly updated database with data from NWIS
    - Regularly updated database with invites sent to potential participants, replies received, participant details and data related to planned and completed visits
- Arranged additional new recruitment group
    - Recruited and set-up additional principal investigators from 4 Welsh hospitals to enable recruitment from additional group of children
    - Gained data related to additional recruitment group from NWIS and added to existing databases
- Recruited and managed nursing research staff
- Provided teaching for research team on testing protocols
- Developed competency packages and provided teaching for nursing staff on all aspects of testing and clinical examination
- Arranged specialist teaching for team members e.g., ARTP course
- Ordered appropriate equipment

Undertaking study

- Arranged mailings for recruitment groups

    - Merging datasets to produce ISAAC questionnaires with RHiNO identification number
    - Produced invite letters, envelope labels and surveys with unique identifier
    - Participated in printing and producing mailing packages with other staff
- Reviewed recruitment numbers and determined optimal mailing strategy with team members
- Obtained medical notes from across Wales and developed and maintained database to collate neonatal medical history
- Undertook home visits alongside other team members
    - Organised visit
    - Contacted GP for lone visitor assessment
    - Took informed parental consent and child assent
    - Performed testing and completed data collection
    - Produced child friendly certificate of participation
    - Entered data onto database
    - Followed-up adverse findings with family and GP
- Performed QC on spirometry tests
- Developed QC process for reviewing spirometry for acceptability and repeatability
- Data cleaning
- Cross referenced results against GLI dataset to ensure % predicted data correct
- Undertook all statistical analysis

End of trial

- Archiving

Summary

This thesis has used community lung function testing in children aged 7 to 12 years to identify and understand persisting lung function deficits in those born preterm (≤34 weeks' gestation) compared with those born at term (≥37 weeks' gestation). I have evaluated spirometry measures, reversibility testing and FeNO measurements. With data from 739 children, 544 preterm- and 195 term-born, it is one of the largest studies of its kind.

I have described how preterm-born children continue to have significant lung function deficits. They also have greater response to 400mcg inhaled salbutamol compared with term-born controls. However, there were no differences in FeNO measurements.

I evaluated the impact that CLD has on childhood lung function and discovered that preterm-born children with and without CLD have significant lung function deficits. Furthermore, exploratory analysis of early life factors identified that IUGR and gestation are significantly associated with childhood lung function deficits, but CLD is not.

Evaluation of children by current %FEV_1 revealed that a significant proportion of children with lung function deficits were not receiving any treatment despite having evidence of a positive bronchodilator response.

Using a combination of current %FEV_1 and FEV_1/FVC I have identified two different respiratory phenotypes: obstructive and non-obstructive. I found that children with the obstructive phenotype have greater lung function deficits, respiratory symptoms, diagnosis of asthma and inhaler use. They also had a greater response to 400mcg salbutamol and a higher proportion of children with a FeNO >35ppb.

I have successfully demonstrated that community lung function testing can assist in identifying children with ongoing lung function deficits and identify those more likely to benefit from inhaled treatments. I propose ongoing community-based surveillance in this vulnerable group of children to improve identification of ongoing lung function deficits and assist in optimising respiratory health.

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Dedication

To Perry, for your unconditional love and support.

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Most importantly, I wish to express my sincere thanks to all the children and families who kindly participated in the study. Without their generosity, this thesis would not have been possible.

List of Publications

Papers

Goulden N, Cousins M, **Hart K**, Jenkins A, Willetts G, Yendle L, Doull I, Williams E M, Hoare Z, Kotecha S (2022) Inhaled Corticosteroids Alone and in Combination with Long-Acting β2 Receptor Agonists to Treat Reduced Lung Function in Preterm-Born Children: A Randomized Clinical Trial, *JAMA Paediatrics,* 176(2):133-141.

Hart K, Cousins M, Watkins W J, Kotecha S J., Henderson A J, Kotecha S (2022) Association of Early Life Factors and Prematurity-Associated Lung Disease: Prospective Cohort Study, *European Respiratory Journal*, 59(5):2101766. doi: 10.1183/13993003.01766-2021. (Appendix 1).

Cousins M, **Hart K**, Gallagher D, Palomino MA, Kotecha S. (2018) Long-term respiratory outcomes following preterm birth. *Revista Medica Clinica Las Condes*; 29(1) 87-97.

Hart K, Cousins M, Kotecha S. (2017) Respiratory outcomes after preterm birth. *Minerva pneumologica*; 56(2):139-151.

Gallacher DJ, **Hart K**, Kotecha S. (2016) Common respiratory conditions of the newborn. *Breathe*;12(1):30–42.

Presentations and posters

Lung function in school-aged children. An ANP's experience of a large research trial and undertaking a PhD. (Oral presentation) *Cardiff and Vale UHB Nursing and Midwifery Conference. November 2019.*

Hart K, Cousins M, Yendle L, Willetts G, Doull I, Williams, M, Kotecha S, Henderson J, Kotecha S (2019) Lung function in school-aged preterm-born children. (Poster) *Cardiff and Vale UHB Nursing and Midwifery Conference*.

Hart K, Cousins M, Willetts G, Yendle L, Doull I, Williams M, Kotecha, S, Henderson J, Kotecha S. (2018) Bronchodilator response in school-aged pre-term born children who had chronic lung disease in infancy. (Poster) *ERS International Conference*.

Hart K, Cousins M, Willetts G, Yendle L, Doull I, Williams M, Kotecha, S, Henderson J, Kotecha S. (2018) Fractional exhaled nitric oxide concentration in school-aged children born with chronic lung disease in infancy. (Poster) *ERS International Conference*.

Awards

BLF & British Paediatric Respiratory Society (BPRS) Travel Fellowship award (2018).

List of Abbreviations

A/N	Antenatal
ARTP	Association for Respiratory Technology and Physiology
ATS	American Thoracic Society
AUC	Area under the curve
AUROC	Area under the receiver operating characteristic curve
BAL	Bronchioalveolar lavage
BD	Bronchodilator
BDR	Bronchodilator response
BMI	Body mass index
BPD	Bronchopulmonary dysplasia
BTPS	Body Temperature and Pressure with Saturated water vapour
BTS	British Thoracic Society
CF	Cystic fibrosis
CI	Confidence interval
CLD	Chronic lung disease of prematurity
CLD_{28}	Chronic lung disease defined as oxygen requirement at 28 days of age
CLD_{36}	Chronic lung disease defined as oxygen requirement at 36 weeks' post-menstrual age in those <32 weeks' gestation or 56 days of age/discharge in those born ≥32 weeks' gestation
COPD	Chronic obstructive pulmonary disease
CT	Computerised tomography
CYARU	Children and Young Adults Research Unit
EIP	Electromagnetic inductance plethysmography
EPT	Extremely preterm
ELBW	Extremely low birth weight
ERS	European Respiratory Society
$FEF_{25-75\%}$	Forced expiratory flow at 25-75% of FVC
$\%FEF_{25-75\%}$	Percent predicted for forced expiratory flow at 25-75% of FVC
FeNO	Fractional exhaled nitric oxide

$FEV_{0.5}$	Forced expiratory volume in the first 0.5 second of a forced expiratory manoeuvre
$FEV_{0.75}$	Forced expiratory volume in the first 0.75 second of a forced expiratory manoeuvre
FEV_1	Forced expiratory volume in the first 1 second of a forced expiratory manoeuvre
$\%FEV_1$	Percent predicted value for forced expiratory volume in the first 1 second of a forced expiratory manoeuvre
FEV_1/ FVC	Ratio between forced expiratory volume in the first 1 second of a forced expiratory manoeuvre and forced vital capacity
FH	Family history
FOT	Forced oscillation technique
FVC	Forced vital capacity
%FVC	Percent predicted forced vital capacity
GLI	Global lung function initiative
ICS	Inhaled corticosteroids
IL	Interleukin
IUGR	Intrauterine growth restriction
IVH	Intraventricular haemorrhage
LABA	Long acting β_2 agonists
LCI	Lung clearance index
LLN	Lower level of normal
LSCS	Lower segment caesarean section
MBW	Multi breath washout
MRI	Magnetic resonance imaging
NEC	Necrotising enterocolitis
NICE	National Institute for Health and Care Excellence
NO	Nitric oxide
NOS	nitric oxide synthase
NWIS	NHS Wales Informatics Service
OR	Odds ratio
P/N	Postnatal
PDA	Patent ductus arteriosus

PEF	Peak expiratory flow
PFT	Pulmonary function test
PGD	Patient Group Direction
PI	Principal investigator
PMA	Post-menstrual age
POM	Prescription only medication
PROM	Prolonged rupture of membranes
PPROM	Premature prolonged rupture of membranes
PT	Preterm
PTC	Preterm-born control
P_{low}	Preterm with %FEV_1 ≤85%
$P_{low(O)}$	Preterm with obstructive respiratory phenotype – defined as %FEV_1 ≤85% and FEV_1/FVC <0.8
$P_{low(NO)}$	Preterm with non-obstructive respiratory phenotype- defined as %FEV_1 ≤85% and FEV_1/FVC ≥0.8
RCT	Randomised controlled trial
RDS	Respiratory distress syndrome
ROC	Receiver operating characteristic curve
ROP	Retinopathy of prematurity
RR	Risk ratio
RV	Residual volume
RV/TLC	Residual volume/total lung capacity ratio
SOP	Standard operating procedure
SR	Systematic review
TC	Term-born control
Th2	T-helper cell type 2
TLC	Total lung capacity
UHW	University Hospital of Wales
WIMD	Welsh index of multiple deprivation

1 Introduction

The survival of preterm-born infants has significantly improved in line with advances in modern health care provision. Despite these improvements in mortality, preterm birth continues to be associated with co-morbidities including pulmonary and neurological dysfunction. Many of these co-morbidities will have life-long impacts on health.

Lung function deficits in the preterm-born population has been extensively studied and there is clear evidence that, whilst those born at the very limits of viability have improved odds of survival, they have the greatest risk of significant long-term lung function deficits (Been et al. 2014). More recent evidence has identified that preterm birth at any gestation is associated with an increased risk of lung function deficits and wheezing in infancy and childhood (Edwards et al. 2015). Long term studies demonstrate that preterm birth has a lasting deleterious effect on lung function which can persist into adulthood (Gough et al. 2012; Doyle et al. 2019).

This chapter will provide an insight into the antenatal and postnatal risk factors for lung function deficits in the preterm-born population, commonly used lung function testing methods and current evidence related to respiratory outcomes in infancy, childhood, and adulthood. It will also detail the hypothesis and specific aims on which my work has been based. I believe that this work will enhance the existing body of knowledge related to the lung function of preterm-born, school-aged children in a community setting.

1.1 Lung development

Lung function and respiratory health are dependent on optimal lung development and growth after preterm birth. Exposure to intrinsic and extrinsic factors may impede this process. Despite lung development extending beyond the antenatal period into early childhood, there is recognition of the association between altered antenatal lung development, impaired neonatal respiratory function, and its negative impact on life-long respiratory health. A comprehensive knowledge of normal lung development in both the antenatal and postnatal period is key to understanding the potential causes of lung function deficits of children born preterm.

Embryological development of the lung is a complex process of structural and vascular development which is dependent on a number of biochemical and molecular processes

(Copland and Post 2004). Knowledge of embryological development of the lungs continues to evolve. However, current understanding classifies lung development into five phases, embryonic (0 - 7 weeks'), pseudoglandular (7 - 17 weeks'), canalicular (17 - 27 weeks'), saccular (28 - 36 weeks') and alveolar (36 weeks' - 2 years) (Joshi and Kotecha 2007) (Figure 1-1).

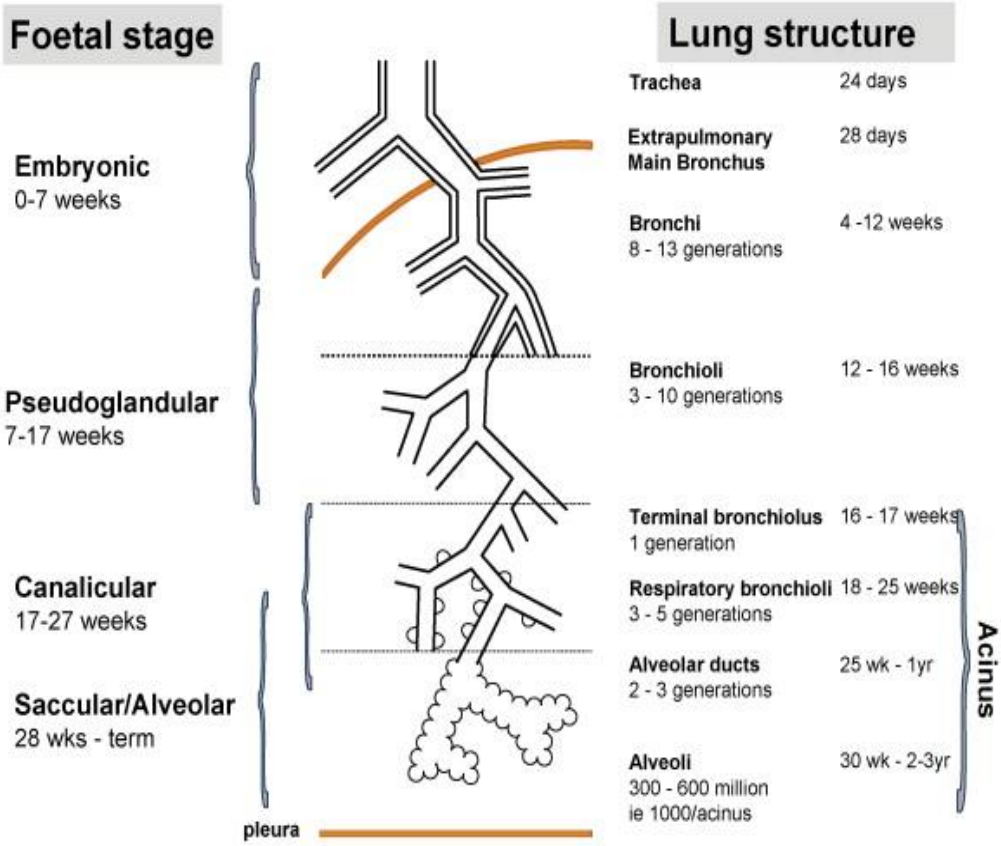


Figure 1-1 Stages of human lung development.
Figure shows the normal stages of development of lung structure alongside gestational age of the fetus. In the first 7 weeks' of pregnancy, lung development begins with the central airway structures including the bronchi which subsequently divide dichotomously. The respiratory units (the acinus) develop from 17 weeks' gestation in the canalicular stage. Development of the peripheral lung structures required for optimal gaseous exchange develop in the saccular/alveolar stages from 28 weeks' of pregnancy and continue beyond birth into infancy.(Joshi and Kotecha 2007) Reproduced with permission.

Development of the large airways and the initial development of pulmonary vascular supply begins early in embryonic life. As early as 3-4 weeks a groove in the ventral surface of the primitive foregut forms. This laryngotracheal groove forms the trachea which subsequently divides into the two main bronchi (Joshi and Kotecha 2007). Branching of the lobar and segmental bronchi stimulated by bronchial mesenchyme is achieved in this stage of development (Alescio and Cassini 1962). Concurrent development of the vascular network that will ultimately comprise of a complex capillary network that surrounds the alveoli, begins as the pulmonary artery arising from a single avascular bud from the 6th pair of aortic arches forming a vascular plexus surrounding the developing lung (Burri 1984). Vasculogenesis and angiogenesis continue in parallel with further lung development.

Further airway division and formation of the conducting airways and terminal bronchioles occurs in the pseudoglandular phase. Cartilage and smooth muscle supporting the airways also develop alongside epithelial cells differentiating from pseudo-stratified cells to columnar and cuboidal cells (Joshi and Kotecha 2007). At the completion of this stage, 20 divisions have occurred and some of the acinar units are formed (Kitaoka et al. 1996).

During the canalicular phase, development of the acinar units are completed - signalling the end of the development of the conducting airways. Concurrent development of the alveolar epithelium incorporating differentiation of epithelial cells into type one and surfactant producing type two pneumocytes alongside angiogenesis of the capillary network that will form the air-blood barrier also occurs (Schittny 2017). Development and enlargement of alveolar epithelium into saccules, increased production of type two pneumocytes and further thinning of the air-blood barrier occurs in the saccular phase (Joshi and Kotecha 2007).

Whilst rudimentary respiratory units are present prior to 36 weeks' gestation, the primary outcome of the alveolar phase is the expansion of a thin lung surface with closely allied thin-walled capillary bed which effectively facilitates optimal gaseous exchange that is vital for biochemical homeostasis. Secondary septation and thinning of the alveolar component of the respiratory unit occurs during this phase. Concurrent fusing of the two capillary layers to form one complete effective network alongside interstitial volume decrease ensures the air-blood barrier is as thin as possible. Whilst this phase begins at 36 weeks' gestation, alveolarisation continues beyond birth into at least infancy. Indeed, recent evidence suggests that this process may continue into late childhood (Narayanan et al. 2013).

During alveolarisation, the number of alveoli increase from hundreds in the antenatal period to several million at birth, to 300 - 600 million in adulthood (Emery and Mithal 1960; Dunnill 1962; Ochs et al. 2004). A final phase of late alveolarisation - where the number of alveoli continues to increase beyond infancy - is described (Emery and Mithal 1960; Davies and Reid 1970); but age-specific evidence is variable. In addition to structural development, growth is ongoing until optimal lung function is achieved in early adulthood. Following a period of stability, loss of function occurs with natural ageing, resulting in a gradual decline in FEV_1 (Fletcher and Peto 1977).

Lung development, growth and ultimately lung function can be influenced by several antenatal and postnatal factors which can impede the attainment of optimal lung function and accelerate the rate of decline in later life. Figure 1-2 details antenatal and postnatal risk factors that can result in abnormal lung growth and development, subsequently resulting in deficits in lung function. A description of the risk factors which can disrupt normal lung development are discussed in the next section.

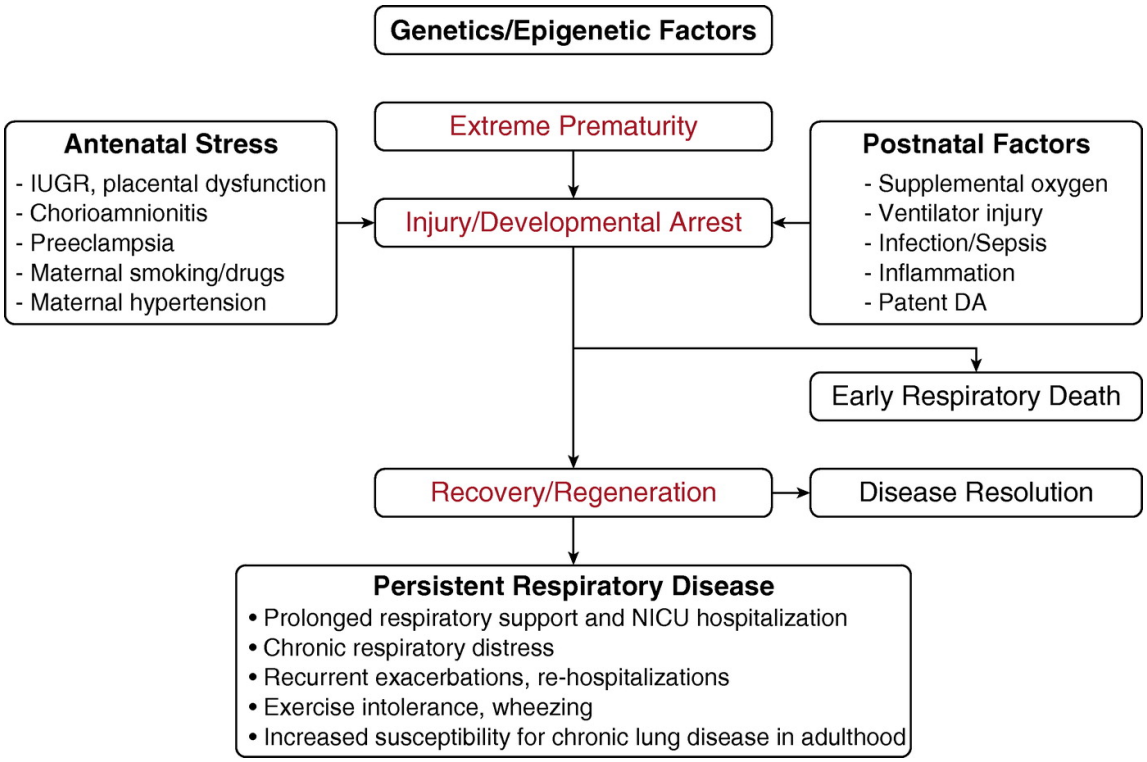


Figure 1-2 Antenatal and postnatal factors associated with lung injury, altered lung development and persistent lung function deficits.
(Taglauer et al. 2018). Reproduced with permission.

1.1.1 Antenatal impact on lung function

The antenatal intra-uterine environment significantly impacts on lung development and future lung function. Organogenesis is impacted by maternal, fetal and environmental factors. Consideration of these factors is vital in understanding the mechanisms of antenatal lung injury.

1.1.2 Maternal health and wellbeing

The health and development of the fetus and placenta is intrinsically linked to the health and wellbeing of the mother. Optimal organogenesis occurs in a stable homeostatic environment within the uterus. Interruptions to the status of the intra-uterine environment can impact on postnatal lung function. It is vital to appreciate the role of maternal health and disease on the antenatal intra-uterine environment and ultimately, the developing fetus.

One factor that can influence the intra-uterine environment is deprivation. Deprivation is associated with poor health outcomes, including greater risk for chronic health conditions and lower life expectancy (Townsend et al. 1992). Deprivation is estimated by comparing factors such as education, income, health, and environment across neighbourhoods. These measures can be used to understand inequalities in health across communities, help develop policies to reduce deprivation, and subsequently improve health outcomes.

The most deprived group of the population have twice the risk for preterm birth compared to the least deprived group (Bonet et al. 2013). Preterm birth at <33 weeks' gestation, chronic lung disease (CLD) at 36 weeks' post-menstrual age (PMA), home oxygen and hospitalisation with respiratory illness were three times higher in those most disadvantaged (Deschamps et al. 2021).

Deprivation has been linked to higher obesity, smoking rates, and air pollution levels (Brunt et al. 2017; Mohammed et al. 2019; Walker and Cresswell 2019). These factors also impact the health and wellbeing of the pregnant mother and her developing fetus.

Obesity and excess weight gain during pregnancy are associated with an increased risk for gestational diabetes, gestational hypertension, pre-eclampsia, low birthweight, and preterm birth (Voerman et al. 2019). Longer term adverse outcomes of maternal obesity include an increased risk for asthma, which can also affect perinatal outcomes (Forno et al. 2014).

Human studies show that maternal antenatal smoking is associated with increased evidence of obstructive lung function deficits (Bisgaard et al. 2009; Balte et al. 2016), increased risk of

wheeze in infancy (De Queiroz Andrade et al. 2020) and asthma diagnosis noted in childhood (Burke et al. 2012). Exposure to antenatal smoke has significant and lasting effects on the fetus' health. Animal studies clearly demonstrate exposure of the fetus to nicotine causes abnormal remodelling of the conducting airways and alveoli (Harding and Maritz 2012). Additional evidence suggests an association between maternal antenatal smoking and modification of genetic expression resulting in elevated risk for asthma in up to two generations underlining the importance of cessation of maternal smoking in pregnancy (Panasevich et al. 2010).

Targeted government policies to reduce smoking rates across the UK have had a positive impact. Legislation banning smoking in enclosed spaces was introduced in Wales in 2007. This smoking ban resulted in a decrease in smoking from 24% to 18% in 2019 (WAG 2019). Rates of maternal antenatal smoking have also reduced. However, a higher proportion of women in Wales continue to smoke during pregnancy (17.8%) compared to England (12.7%) and Scotland (13%) (PHE 2019; PHW 2020; ScotPHO 2021). Furthermore, rates continue to be higher in the three most deprived quintiles compared to those in the 2 least deprived quintiles (WAG 2019).

Further exposure to environmental pollution has been associated with low birth weight/intra-uterine growth restriction (IUGR), impaired lung growth and increased respiratory illness (Melody et al. 2019; Sly et al. 2021).

1.1.3 Fetal health and wellbeing

Optimal growth and development of the fetus is fundamental to organogenesis. Alterations to normal growth patterns potentially increase the risk of dysgenesis and associated long-term adverse health outcomes.

Placental insufficiency - most commonly due to pregnancy associated pre-eclampsia - can have a profound impact on the growth of the fetus leading to IUGR and preterm birth. Approximately 3-5% of pregnancies are affected by pre-eclampsia (Bokslag et al. 2016). Whilst it cannot be resolved without removing the placenta and delivery of the fetus, preventative measures including targeted health promotion to address contributing factors such as obesity have been introduced to reduce the risk for developing pre-eclampsia. Greater fetal monitoring and the medical management of symptoms with labetalol, hydralazine and magnesium have assisted in protecting the health of the mother and fetus, including reducing rates of preterm delivery (ACOG 2015). However, antihypertensive

medication use can be associated with growth restriction in the fetus (von Dadelszen and Magee 2002).

Maternal antenatal smoking may be protective from pre-eclampsia (Iacobelli et al. 2017). However, both maternal antenatal smoking and environmental pollutants are associated with poor fetal growth (Abraham et al. 2017; Huang et al. 2019).

There is clear evidence to suggest that impaired growth affects lung development but understanding of the mechanisms behind this phenomenon is predominantly based on animal studies. Studies in sheep demonstrate that induced IUGR leads to altered structural development of the lungs - with fewer, larger alveoli, thicker inter-alveolar septa, a thickened blood-air barrier, and a surface area that is up to 10% smaller (Maritz et al. 2004). Dysfunction of surfactant synthesis and production have also been described in growth restricted fetal mice (Bahner 2004). Evaluation of intrauterine growth and childhood wheeze in a cohort of children in the UK demonstrated increased odds of wheeze-ever in children with evidence of deceleration in growth between the first and second trimester (Lowe et al. 2017). Conversely, accelerated growth between the second and third trimester was also associated with childhood wheeze (Lowe et al. 2017). Alterations to growth in both the first and second trimester may impact on the development and growth of the conducting airways leading to an imbalance between development and growth of the airways compared to lung size. Initially described by Green et al, pulmonary dysanapsis occurs when growth of the respiratory system results in disparate growth between airway calibre and lung size (Green et al. 1974), thus an imbalance between airway calibre and lung size. Despite having a forced expiratory volume in the first second (FEV_1) within normal limits, significant discordance in the growth of airways compared to lung size are attributed to obstructive patterns of lung function deficits. A study of preterm-born adults showed that they had a lower dysanapsis ratio - suggestive of smaller airway calibre compared with lung volume - when compared to term-born controls. In the preterm-born group, those with CLD had the lowest ratio. The authors report concordance between these findings and lower rates of expiratory flow at rest and during exercise and suggested that greater dysanaptic growth in preterm-born adults was contributing to airflow obstruction (Duke et al. 2018).

IUGR is associated with higher risk of fetal death and multi-organ compromise - including respiratory compromise - in the neonatal period for those who survive to delivery (Engineer and Kumar 2010). Barker's hypothesis of fetal 'programming' describes the lasting negative impact that impaired intrauterine growth and development has beyond the neonatal period

into adulthood (Barker 2004). Evidence supporting this theory includes the significantly lower spirometry observed in children aged 8 - 9 years, born with IUGR, compared to those born without IUGR (Kotecha et al. 2010). Additional evidence from a longitudinal study shows a clear correlation between low birth weight, weight at 1 year, and increased mortality from chronic obstructive airways disease in adulthood (Barker et al. 1991) .

In summary, the impact of antenatal growth and development has a lasting impact on an individual's respiratory health and wellbeing. Strategies to ensure optimal steady growth - such as optimising maternal nutrition and smoking cessation - are essential to reducing the long-term impact of poor respiratory health.

1.1.4 The intra-uterine environment

The correlation between intra-uterine infection/inflammation and fetal or early neonatal death is well documented (Barton et al. 1999). Chorioamnionitis - the inflammation of fetal membranes, chorion and amnion - is the most common presentation of intrauterine inflammation and often associated with infection. It is identified as a primary cause of preterm birth, with an inverse relationship between the incidence of intra-uterine infection and gestational age at birth (Goldenberg et al. 2000; Lahra et al. 2009).

Whilst infective organisms are not always isolated, *Ureaplasma* - a genital mycoplasma - has been consistently associated with chorioamnionitis (Abele-Horn et al. 2000). The association between pulmonary *Ureaplasma* colonisation and development of CLD has also been described. In their meta-analysis, Wang et al found the risk ratio (RR) in infants with *Ureaplasma* colonisation for developing CLD was 1.72 (95% CI 1.5 to 1.96) times that of neonates who were not colonised (Wang et al. 1995). Lowe et al also described how neonates with evidence of pulmonary *Ureaplasma* colonisation were at an increased risk for CLD at 36 weeks' PMA (OR of 2.22, 95% CI 1.42 to 3.47 (Lowe et al. 2014). A more recent meta-analysis of perinatal outcomes of mothers with evidence of *Ureaplasma* infection also found a positive association with CLD at 36 weeks' PMA (OR 2.39, 95% CI 1.73 to 3.30) (Xu et al. 2022).

This suggests that injury to the developing lung may be compounded by lung inflammation that commences in-utero. Despite this, only 47% of 167 neonatal units across Europe routinely test for *Ureaplasma* (Pansieri et al. 2014).

In recognition of the potential role of antenatal maternal *Ureaplasma* colonisation, current UK guidance for prolonged preterm rupture of membranes (PPROM) - often associated with preterm delivery and chorioamnionitis - includes a 10-day course of Erythromycin prior to delivery (RCOG 2019).

The potential use of macrolides in neonates to reduce lung inflammation caused by *Ureaplasma* and subsequent CLD begin in the 1990's. However, the evidence remains limited. Erythromycin, Clarithromycin and Azithromycin have all been tested for their efficacy in reducing CLD in those who have evidence of *Ureaplasma*. However, the majority of these studies were small and therefore, underpowered (Mabanta et al. 2003). Some studies used culture rather than more rapid and sensitive PCR to identify those colonised with *Ureaplasma*, resulting in delays in commencing treatment. More recently, a meta-analysis has shown that prophylactic Azithromycin is associated with reduced incidence of CLD. However, the authors acknowledge the lack of robust pharmacokinetic knowledge associated with use in the neonatal population and suggest caution in routine use (Nair et al. 2014). There is further evidence demonstrating that prophylactic Azithromycin significantly reduces the incidence of CLD (RR=0.83, 95% CI 0.71 to 0.98, p=0.02). Whilst the safety profile was better than Erythromycin, the authors suggest further safety and adequately powered studies efficacy are required (Smith et al. 2015). It is hoped that the recently completed AZithromycin ThErapy for Chronic Lung Disease of Prematurity (AZTEC) study will identify if early Azithromycin treatment reduces the incidence of CLD (Lowe et al. 2020).

The association between chorioamnionitis and accelerated lung maturation is well documented (Watterberg et al. 1997; Bachurski et al. 2000). The reported impact of this maturation process is mixed. Whilst several authors suggest that chorioamnionitis reduces the incidence of respiratory distress syndrome (RDS) (Watterberg et al. 1996; Lahra et al. 2008) more recent studies suggest that this benefit is limited to infants with mild to moderate chorioamnionitis (Park et al. 2015). Been et al identified that infants with severe chorioamnionitis have a reduced response to exogenous surfactant, demonstrated by higher oxygen requirements and longer periods of ventilation compared to those with lower levels of chorioamnionitis, both of which lead to further lung injury (Been et al. 2010).

Whilst exposure to chorioamnionitis may reduce the risk of RDS, several authors suggest an increased incidence of CLD in infants exposed to chorioamnionitis (Dessardo et al. 2012; Kunzmann et al. 2013).

The correlation between lung inflammation and persisting lung injury in the form of CLD is well evidenced. Elevated interleukin (IL) IL-1β and Thromboxane B2 have been identified in tracheal aspirates in preterm-born infants exposed to chorioamnionitis who subsequently developed CLD (Watterberg et al. 1996). Increased levels of pro-inflammatory cytokines IL-6 and chemokines such as IL-8 have also been described in postnatal broncho-alveolar lavage (BAL) fluid of infants who subsequently develop CLD (Chakraborty et al. 2013). Preterm-born children with impaired lung function have been noted to have increased neutrophils and its chemoattractant IL-8 in induced sputum - suggesting inflammation continues beyond infancy (Teig et al. 2012).

Kunzmann et al suggest that alongside signalling pathway modification and subsequent adaptation in lung development and function in those exposed to chorioamnionitis, infants exposed to in-utero inflammation develop abnormal adaptations to their immune system resulting in reduced ability to respond to subsequent infections (Kunzmann et al. 2013). This maladapted immune response may explain the evidence of ongoing and persistent lung inflammation in children with CLD and the increased incidence of hospital re-admissions seen in preterm-born infants (Pramana et al. 2011; Berard et al. 2012).

The increased risk of preterm birth associated with chorioamnionitis compounds the impact that in-utero infection and inflammation has on the fetus in terms of remodelling of the respiratory and immune system. Whilst the longer-term impact of chorioamnionitis on respiratory function and the development of CLD is less clear, ongoing lung inflammation is observed in those with CLD which may result in impaired lung function in the long-term.

1.2 Prematurity and respiratory consequences of preterm birth

Preterm birth and postnatal health care interventions contribute to further lung injury and consequent later lung function deficits. There have been significant changes in the management of preterm labour and postnatal management strategies which have resulted in greater survival rates of the preterm-born infant. However, the impact of these changes on postnatal lung development and function are less clear.

1.2.1 Causes of preterm birth

Worldwide rates of preterm births are poorly documented. In 2010, an estimated worldwide figure of 15 million children were born below 37 weeks' gestation (Blencowe et al. 2012).

Equalling approximately 11% of all live births, this number is noted to be increasing (Blencowe et al. 2013). Prematurity is the leading cause of death in all children under 5 years. Approximately 1 million preterm-born children die each year due to complications of prematurity (Liu et al. 2016). Complications related to preterm birth are inversely related to gestational age and whilst outcomes for the extremely preterm-born have improved, morbidity has remained static (Saigal and Doyle 2008; Costeloe et al. 2012). The World Health Organisation has defined sub-categories of prematurity – extremely preterm (<28 weeks), very preterm (28 to <32 weeks) and moderate to late preterm (32 to <37 weeks) (WHO 2012). Late preterm birth has also been defined as 34 to 37 weeks' gestation (Engle 2006).

The reasons for preterm birth are complex but can be separated into two broad categories - spontaneous delivery and provider-initiated delivery (Goldenberg et al. 2012). Blenclowe et al outline the risk factors for spontaneous delivery including maternal age (adolescent or older age), infection, multiple pregnancy, chronic maternal medical conditions, maternal lifestyle and nutrition, and genetic factors. They also describe how maternal or fetal health concerns are the greatest cause of provider-initiated deliveries (Blencowe et al. 2013).

Antenatal interventions to improve maternal health, the use of antibiotics to reduce the risk of chorioamnionitis in cases where there is high risk and medications to prevent preterm labour have all been welcomed to reduce the incidence of preterm delivery.

1.2.2 Respiratory consequences of preterm delivery

The consequences of preterm birth can impact on short-, medium- and long-term respiratory health. Neonatal respiratory distress syndrome (RDS) occurs shortly after birth and affects short- and medium-term respiratory health. Children with longer-term respiratory insufficiency are diagnosed with chronic lung disease (CLD). These two respiratory diseases will be described in greater detail.

1.2.2.1 RDS

The initial challenge for the new-born infant is to establish homeostasis. To achieve this, optimal gaseous exchange must occur via the respiratory system. RDS is characterised by pulmonary insufficiency and respiratory compromise due to surfactant deficiency. It is classically accompanied by a ground glass appearance of the lung fields on chest x-ray (Sweet et al. 2013). Left untreated it can result in death. Largely associated with preterm-born

children, the risk of RDS is inversely proportional to gestational age with infants born prior to 28 weeks' gestation being the most vulnerable. Born during the canalicular phase of lung development and only having primitive respiratory units - they have insufficient numbers of surfactant producing type two pneumocytes, thickened alveolar walls and underdeveloped capillary networks - leading to the need for respiratory support and oxygen which in turn causes further injury to the developing lung.

Preventative strategies to reduce the incidence and severity of RDS include antenatal maternal steroid administration and prophylactic neonatal instillation of exogenous surfactant have become standard practice in the management of preterm birth (Sweet et al. 2019). Antenatal maternal administration of glucocorticoids has been demonstrated to be efficient in reducing the incidence of RDS and some co-morbidities associated with preterm delivery, although the long-term impact remains unclear (Roberts et al. 2017). Postnatal administration of exogenous surfactant to decrease surface tension in the respiratory units has been shown to reduce mortality and air leak in preterm-born infants (Suresh and Soll 2005), although the optimal timing and method of administration is currently under review (Sakonidou and Dhaliwal 2015; Wheeler et al. 2015).

Whilst both ventilation and supplemental oxygen therapy to support respiratory function in the preterm-born infant have been recognised as necessary, animal studies have demonstrated they cause acute lung injury to the developing lung (Warner et al. 1998; Albertine et al. 1999). Technological advances in the last 20 years have enabled clinicians to employ lung protective strategies to reduce lung injury caused by essential respiratory support. Changes to management strategies since 2000 include avoidance of hyperoxia through judicious use of oxygen and the use of non-invasive methods of ventilation where possible in order minimise exposure to injurious, invasive ventilation (Sakonidou and Dhaliwal 2015). For those who require invasive ventilation, lung protective strategies including permissive hypercapnia and volume targeted ventilation are used to reduce the risk of further lung injury which can lead to the development of CLD (Wheeler et al. 2011; Sweet et al. 2019) .

1.2.2.2 CLD - Bronchopulmonary dysplasia (BPD)

Northway et al described a new respiratory disease initially called BPD, otherwise known as CLD, in 1967. It was characterised by marked pulmonary fibrosis, severe epithelial damage, heterogenous areas of atelectasis and hyperinflation, and hyperplasia of the airway smooth

muscle with associated hypertension of the pulmonary vasculature noted on radiological and pathological assessment (Northway et al. 1967). This disease was observed in moderately preterm-born infants who had received respiratory support from rudimentary ventilators and high concentration oxygen.

More recently, Jobe described a “new” form of CLD observed in infants born at much lower gestational ages and characterised by altered lung architecture with evidence of dysfunctional alveolar-capillary development, larger and fewer alveoli and greater interstitial fibrosis (Jobe 1999). Up to 75% of children born at <26 weeks’ gestation are diagnosed with this “new” form of CLD (Costeloe et al. 2000). Using baboon models of CLD, Coalson et al demonstrated the architectural changes associated with “new” CLD compared to term-born equivalent lung development (Figure 1-3).

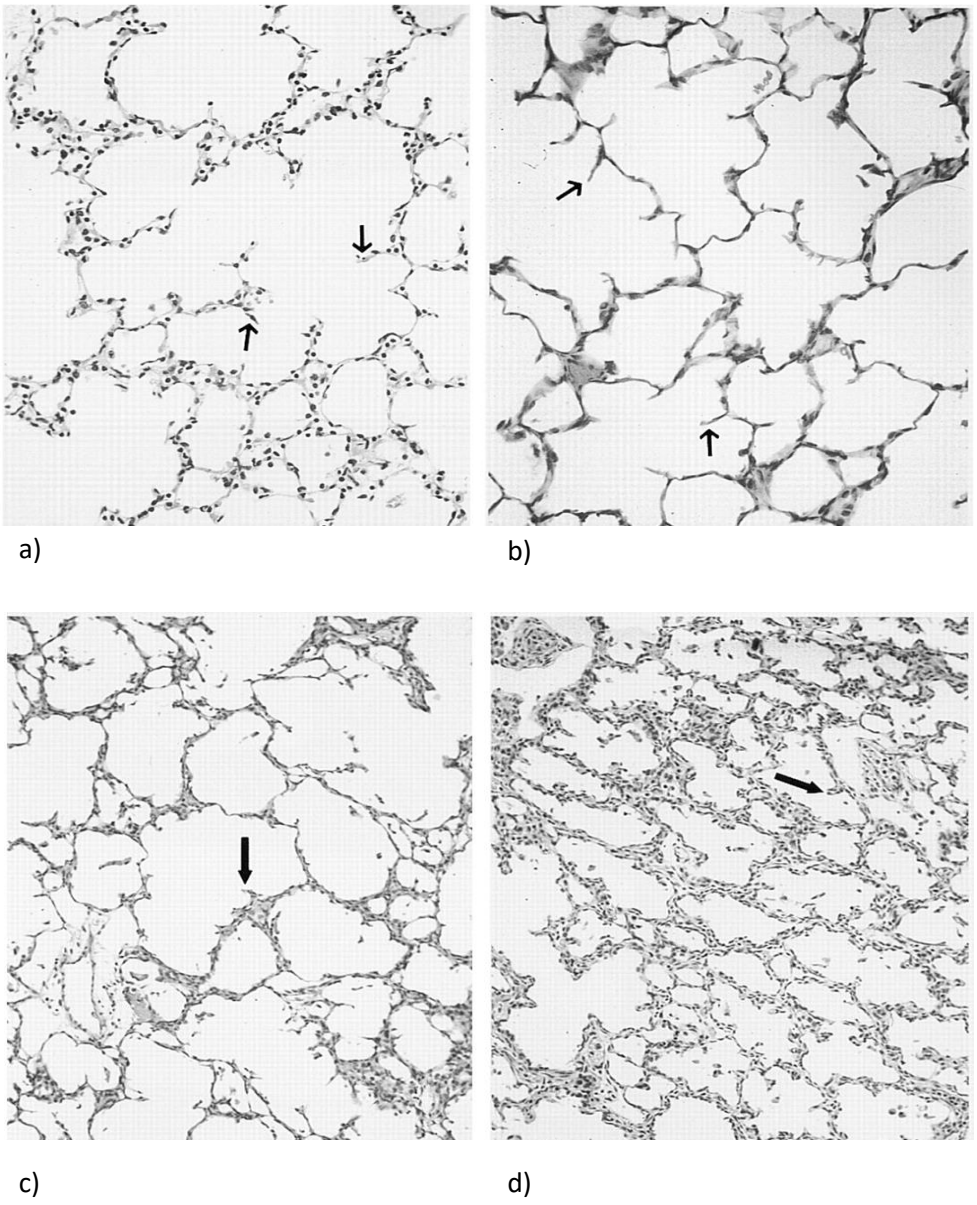


Figure 1-3 Histological samples detailing architectural lung development in preterm-born baboons with CLD compared with controls.

Control specimens a) and b). a) Lung at term with thin alveolar walls. Presence of secondary crests and alveoli (arrows), b) Lung tissue at 2 months of age with thin saccular and alveolar walls and fewer nuclei in alveolar walls. Thinned secondary crests and distinct alveolar walls shown (arrows). Preterm-born CLD specimens c) and d). c) Lung at 30 days of age with increased interstitial change and minimal secondary crests (arrows). d) 39 days of age with severe change evident with minimal secondary crests (arrow). Adapted from (Coalson et al. 1999). Reproduced with permission.

The causes of this "new" CLD in preterm-born children born in the post-surfactant era are multi-factorial and probably due to both antenatal and postnatal factors - all of which can lead to the characteristic abnormal lung structure (Figure 1-4). The overriding influencing factor for CLD is the degree of prematurity or, more likely, the phase of lung development at delivery of the preterm-born infant.

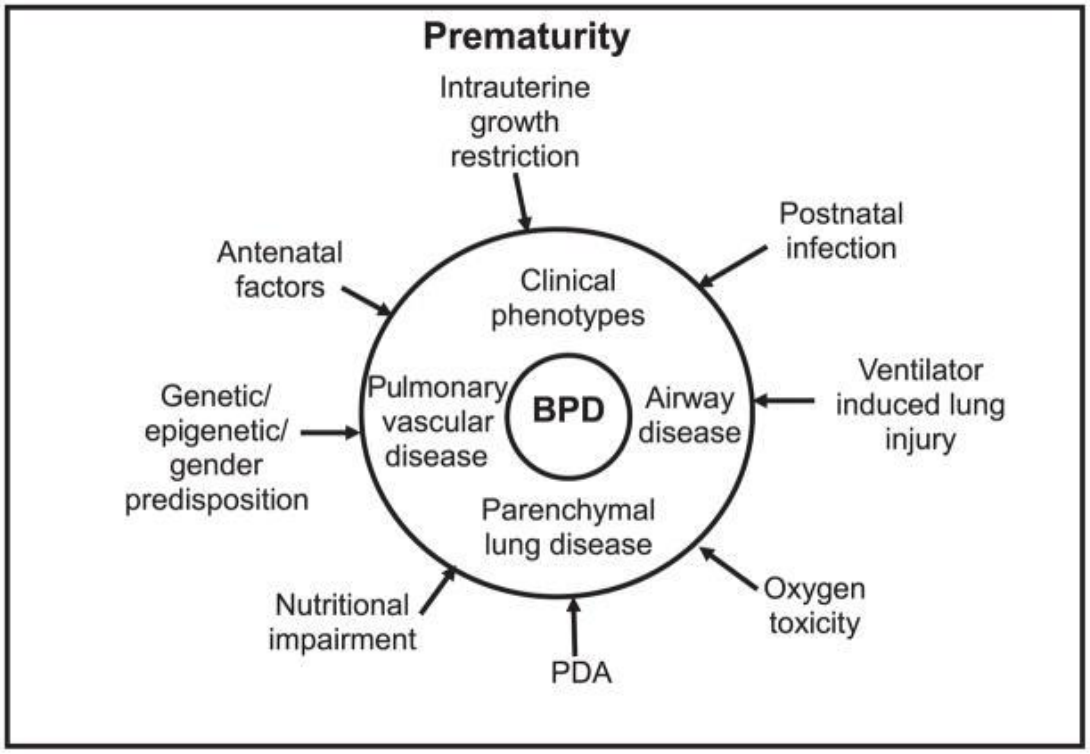


Figure 1-4 Antenatal and postnatal factors associated with CLD.
Largest influencing factor is degree of prematurity (Higgins et al. 2018). Reproduced with permission.

Antenatal intra-uterine inflammation and IUGR have been associated with maladapted organogenesis resulting in the interruption of alveolar and pulmonary vascular development (Maritz et al. 2004; Dessardo et al. 2014; Lardon-Fernandez et al. 2017). Postnatal lung damage and the development of CLD secondary to mechanical ventilation and supplemental oxygen therapy have been described in both animal models and human studies (Coalson et al. 1995; Coalson et al. 1999; Rutkowska et al. 2018). Additional risk factors include patent ductus arteriosus (PDA), retinopathy of prematurity (ROP) and intraventricular haemorrhage (IVH) (Lardon-Fernandez et al. 2017). As expected, these risk factors often indicate poor neonatal health and are more commonly observed in those born at lower gestational ages.

The definitions to diagnose CLD have evolved. Northway et al used clinical, radiological, and pathological observations to describe a four-stage disease progressing from initial stage of acute respiratory distress to more chronic disease state observed at one month of age (Northway et al. 1967). Bancalari et al developed diagnostic criteria based on observed associations between oxygen requirements at 28 days of age, the need for positive pressure ventilation in the first week and radiological findings (Bancalari et al. 1979). Whilst these criteria identified clinically useful characteristics of CLD, their association with long-term outcomes were not evaluated. Subsequently, Shennan et al demonstrated that, whilst oxygen requirement at 28 days of age was a good predictor in those born >30 weeks' gestation, oxygen requirement at 36 weeks' PMA had a greater positive predictive value for abnormal pulmonary findings at 2 years in those born <1500grams (Shennan et al. 1988).

The value of oxygen supplementation for 28 days of age and 36 weeks' PMA was further assessed during the development of NICCHD consensus guidelines in 2001. Data showed that oxygen for 28 days of age was useful for predicting oxygen dependency at 36 weeks' PMA, whilst assessment at 36 weeks' PMA was useful for predicting those requiring oxygen at the time of discharge. The prediction of rehospitalisation with respiratory disease and medication use following discharge was similar for oxygen for 28 days of age and at 36 weeks' PMA. Subsequent diagnostic criteria within the guidelines used supplemental oxygen for at least 28 days of age to diagnose CLD, and oxygen requirement at 36 weeks' PMA to characterise the severity of the disease (Jobe and Bancalari 2001). Thus, the use of mild, moderate, and severe classifications for CLD was established. Table 1-1 details these diagnostic criteria.

	Birth at <32 weeks' gestation	**Birth at ≥32 weeks' gestation**
Time point of assessment	36 weeks' PMA or discharge	>28 days of age but <56 days of age or discharge
	Treatment with oxygen >21% for at least 28 days plus;	
Mild CLD	Breathing room air at 36 weeks' PMA or discharge	Breathing room air by 56 days or discharge
Moderate CLD	Need for <30% oxygen at 36 weeks' PMA or discharge	Need for <30% oxygen at 56 days or discharge
Severe CLD	Need for ≥ 30% oxygen and/or positive pressure at 36 weeks' PMA or discharge	Need for ≥ 30% oxygen and/or positive pressure at 56 days or discharge

Table 1-1 Diagnostic criteria for presence and severity of CLD.

Criteria for assessing for the presence and severity in infants born <32 and ≥32 weeks' gestation. The presence of CLD is determined by the requirement of oxygen for 28 days in all infants. Severity of CLD is assessed at 36 weeks' PMA in those <32 weeks' and at 8 weeks' PMA in those ≥32 weeks' gestation. In both groups severity is assessed as mild – requiring no supplemental oxygen or respiratory support, moderate – requiring <30% supplemental oxygen, severe – needing higher levels of supplementary oxygen, and or mechanical respiratory positive pressure. (Jobe and Bancalari 2001).

Whilst these criteria may be useful in clinical practice, they are based on limited evidence and have not been evaluated against long-term respiratory outcomes. Additional restriction of diagnostic assessment criteria to one treatment modality ignores the importance of the underlying disease pathologies and advances in treatment options. Thus, leading to imprecise criteria which are subject to individual interpretation and variations in practice.

Higgins et al evaluated diagnostic criteria for CLD against adverse respiratory and

neurological outcomes at 18-26 months. They suggest diagnosing CLD and assessing of disease severity at 36 weeks' PMA by using criteria which include radiological evidence of parenchymal lung disease and different types of respiratory support, including oxygen requirements (Higgins et al. 2018). Jensen et al go further, advocating the removal of oxygen-dependency from all diagnostic criteria and assessment of CLD in preference for mode of respiratory support (Jensen et al. 2019).

Whilst these more recent methods of diagnosing and assessing disease severity account for advances in available methods of respiratory support, they also have limitations. Like Shennan et al, Higgins et al attempted to provide a clearer link between diagnosis and health outcomes. However, they did not address outcomes beyond infancy and their assessment criteria continue to be subject to variability in clinical practice. Jensen's approach has been criticised as being too simplistic (Bancalari et al. 2019). To date, these newer definitions to diagnose CLD have not been integrated into clinical practice.

CLD has routinely been used as a predictor for longer-term lung function deficits. Current consensus supports the theory that those with CLD are at highest risk for persisting lung function deficits in the future than those without CLD. However, evidence supporting this theory is contradictory. In a longitudinal, study Mello et al. showed that, whilst CLD survivors had initial differences in pulmonary mechanics compared to preterm-born children without CLD, these resolved by 8 months of age (Mello et al. 2015). Conversely, Ronkainen et al demonstrated that preterm-born children have lower lung function than term-born controls, with infants diagnosed with moderate and severe CLD having the greatest deficits (Ronkainen et al. 2015). They also suggest that those with mild CLD may recover function to a level comparable with preterm-born infants who did not have CLD. These contradictory findings raise two questions: does the current definition of CLD predict long-term respiratory outcomes, and is the severity of disease relevant?

In a systematic review (SR) of large multi-centred randomised controlled trials (RCT) between 2000 and 2015, Corwin et al evaluated the value of CLD as a surrogate marker for respiratory outcomes at 2 years. They found that CLD did not consistently predict long-term respiratory outcomes. They also identified highly variable definitions for CLD and different respiratory outcomes between available studies (Corwin et al. 2018). These findings suggest that the predictive value of CLD may be limited.

Advances in medical care have resulted in improved respiratory outcomes for moderately preterm-born infants in the neonatal period. However, it is increasingly recognised that they are also at risk of long-term poor respiratory outcomes (Kotecha et al. 2012a). Whilst there have been attempts to adapt diagnostic criteria to reflect the evolution in CLD, they are limited in their correlation with longer-term outcomes. With the value of CLD as a predictor for longer-term lung function deficits uncertain, it is reasonable to suggest that a reappraisal of the predictive value of CLD - including classification of disease severity - is required.

In the literature, the terms BPD and CLD are often used interchangeably. For clarity in this thesis, I shall use CLD when referring to preterm-born infants with BPD/CLD.

1.3 Techniques for assessing lung function

Injurious exposures in both the antenatal and postnatal period can contribute towards impaired organogenesis and maladaptation of organ or system functionality. However, ongoing lung development beyond the neonatal period may offer the prospect of recovery from injury and continuing improved respiratory health. Thus, to provide accurate information relating to lung function in the preterm-born population it must be evaluated beyond the neonatal period. This section explores commonly used lung function methods. Evidence related to lung function in the preterm-born population will be discussed separately.

1.3.1 Spirometry

Invented in the 1840's by surgeon John Hutchinson, Spirometry is an accessible and reliable tool for assessment of both static (volume) and dynamic (flow) lung function that is routinely used in both community and hospital settings. Figure 1-5 identifies common spirometry measurements.

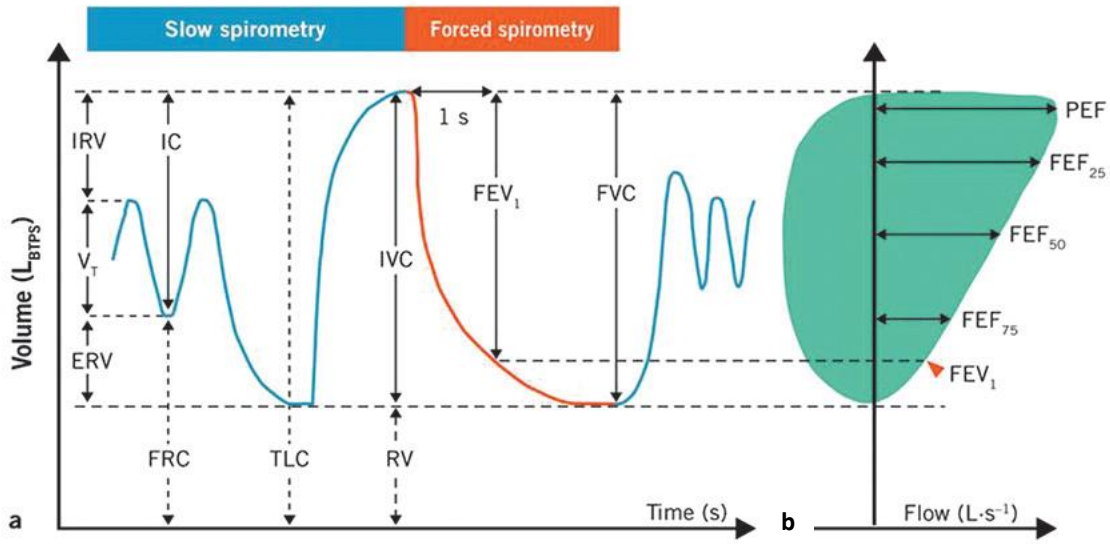
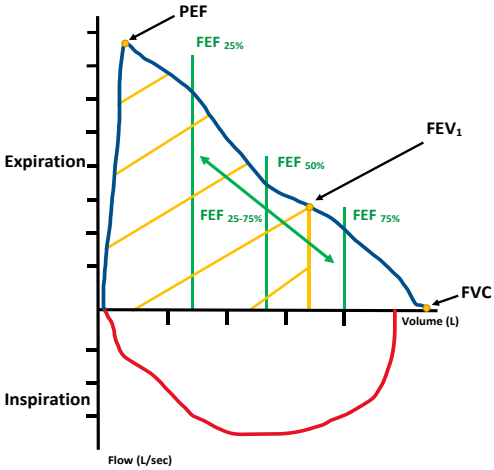


Figure 1-5. Common spirometry measures.
a) Lung volume and time; Slow spirometry - IVC inspiratory volume capacity: including Inspiratory reserve (IR), Expiratory reserve volume (ERV) and tidal volume (V_t), Forced spirometry – Forced expiratory volume in 1 second (FEV_1), Forced vital capacity (FVC); b) Flow-volume curve during forced expiration - Peak expiratory flow (PEF), Forced expiratory flow (FEF) - measured at 25, 50 and 75% of FVC and are reflective of flow in smaller airways. (Criee et al. 2015) (Reproduced with permission).

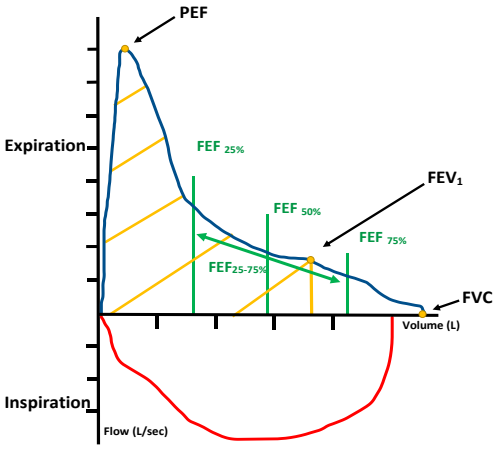
Performance of a forced expiratory manoeuvre enables assessment of dynamic lung function by examining the relationship between volume and time and between flow and volume. Measurement of forced expiratory volume in the first 1 second (FEV_1) of a forced expiratory manoeuvre is helpful in assessing airways that are greater than 2mm in size (Vogt et al. 2014). More importantly, it is used to identify airflow limitation in children and adults. Due to the relatively large airways compared to lung volume, forced expiratory volume in the first 0.5 second ($FEV_{0.5}$) and the volume in the first 0.75 second ($FEV_{0.75}$) of a forced expiratory manoeuvre are advocated for use in pre-school children (Aurora et al. 2004b).

Forced vital capacity (FVC) is used to identify those with reduced lung volumes. A disproportionate reduction in FEV_1 compared to FVC - by calculating the FEV_1/FVC ratio - is used to identify those with obstructive lung function deficits. In adults, a ratio of <0.7 is used in the diagnosis of obstructive diseases such as asthma; and a ratio of ≥0.7 used to diagnose restrictive diseases such as pulmonary fibrosis (NICE 2017a). Currently, there are no established parameters for FEV_1/FVC ratio in children. Guidelines for assessment and diagnosis of asthma in children suggest utilising the adult FEV_1/FVC ratio (NICE 2017a). However, the well documented physiological differences in respiratory function between

children and adults and lack of evidence base for using this ratio and has resulted in the guidance being heavily criticised (Murray et al. 2017). Conversely, those with a normal ratio may have lung function deficits which are restrictive in nature. Assessment of total lung capacity (TLC) by body plethysmography is the gold standard test for formally diagnosing restrictive lung function deficits (Pellegrino et al. 2005). Some spirometry measures have been suggested as potential surrogates for TLC in the adult population (Vandevoorde et al. 2008; D'Aquino et al. 2010). However, these have limited validity and are not useful in the paediatric setting. Whilst spirometry cannot formally diagnose restrictive lung function deficits, additional inspection of the flow/volume loop alongside the ratio helps identify those with obstructive and restrictive patterns of lung function deficits (Figure 1-6).



a)



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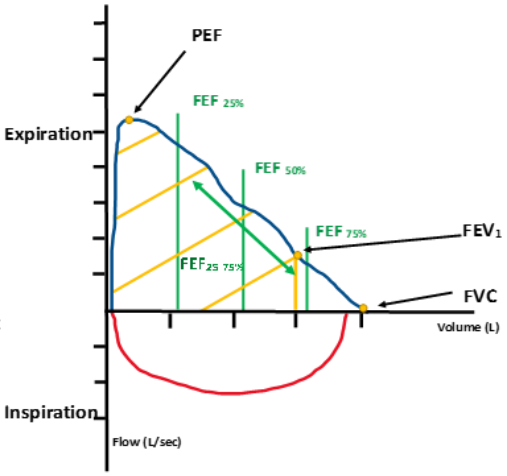


c)

Figure 1-6 Flow volume loops

Demonstrating normal, obstructive, and restrictive patterns of forced spirometry. a) Normal flow/volume curve, b) Obstructive flow/volume curve; concave appearance of expiratory manoeuvre resulting in reduced FEV_1 and forced expiratory, whilst FVC remains normal, c) Restrictive pattern; all measures are reduced. Adapted from (Marchn 2014). Reproduced with permission under Creative Commons Attribution (CC BY) licence.

Like all lung function tests, spirometry has limitations. The use of consistent guidelines for children, recognised education programs for professionals performing the tests and stringent quality control mechanisms contribute to valid and reliable spirometry measures (Graham et al. 2019).

Performance of high-quality spirometry is dependent on the cognitive and physical ability of the participant. Thus, it is not very practical in very young children. One study showed 68% of children between 5 and 8 years were successful at performing spirometry (Gochicoa-Rangel et al. 2013). A further study of 399 children showed 74% of children between 4 and 17 years successfully performed acceptable and repeatable spirometry, with competency increasing with age (Loeb et al. 2008). Incentives to aid performance have been used in pre-school children, although their value in school-aged children is less clear (Aurora et al. 2004b).

The most common reason for failure to perform of spirometry in children is the inability to perform a complete FVC. Failure to perform an acceptable end of test by not achieving plateau and early termination accounted for over 50% of reasons for failure (Loeb et al. 2008).

Whilst FEV_1 reflects the larger airways, there is a growing appreciation that assessing smaller airways may assist in identification of early respiratory disease. Forced expiratory flow at 25-75% of FVC ($FEF_{25–75\%}$) is thought to reflect the patency of the smaller airways. However, calculation of $FEF_{25–75\%}$ is dependent on the FVC and small changes in FVC can lead to high variability in $FEF_{25–75\%}$. Thus, $FEF_{25–75\%}$ needs to be viewed with caution. Other tests such as multi breath washout (MBW), lung clearance index (LCI) and forced oscillation technique (FOT) can assess smaller airways more accurately. However, Simon et al suggest that in children, $FEF_{25-75\%}$ correlates well with bronchodilator response, and may provide useful information to supplement FEV_1 and FVC findings (Simon et al. 2010).

1.3.2 Reversibility

The use of reversibility testing to determine the impact that bronchodilator administration has on observed air-flow limitation is commonplace in lung function testing. Administration of β_2-agonist medication - such as salbutamol - enables assessment of response to medication and degree of reversibility of airway obstruction. Whilst there is guidance on what constitutes a positive bronchodilator response (BDR), especially for adults (Pellegrino et al. 2005), current guidance also allows individual clinicians to determine their own choice

of medication and dosage (Miller et al. 2005). Whilst this may be reasonable in clinical practice, it has led to a high level of variability in choice of medication and administered dose in reported clinical studies – limiting comparison of BDR across studies, especially in small populations such as preterm-born children. Furthermore, the degree of improvement that constitutes a positive BDR is unclear.

1.3.3 Body plethysmography

Body plethysmography enables the collection of lung function measures that include both static and dynamic lung volumes, and airway resistance. Comprising of a volume-constant box which encompasses the whole body, volume changes during breathing manoeuvres are measured by sensors within a mouthpiece and the box itself. Flow during breathing manoeuvres is also measured by conventional equipment (pneumotachograph) within the mouthpiece.

Key body plethysmography measures include intrathoracic gas volume and airway resistance. Measurement of inspiratory vital capacity (IVC) and expiratory reserve volume (ERV) enable calculation of residual volume (RV) and total lung capacity (TLC). In addition to TLC being the gold standard for diagnosing restrictive lung function deficits, RV/TLC is helpful in the assessment of hyperinflation often observed in obstructive disorders such has asthma (Tiwari et al. 2017). Additional assessment of airway narrowing associated with obstruction can also be undertaken with airway resistance measures.

1.3.4 Multi-breath washout (MBW) and lung clearance index (LCI)

MBW and calculation of LCI are used in both cystic fibrosis (CF) and asthma to assess ventilation inhomogeneity and the peripheral airways. Analysis of the washout of an inert tracer gas from the lungs during stable tidal breathing enables calculation of both lung volume and ventilation inhomogeneity. LCI is a calculated value, based on the number of turnover breaths required to clear the lung of the inert gas. An elevated LCI reflects ventilation inhomogeneity. Additional measurements of the conducting airways (S_{cond}) and lung acinus (S_{acin}) have also been used to assess reginal homogeneity.

LCI is routinely used in preference to spirometry in those with CF with evidence that is the optimal test for detecting early lung disease (Aurora et al. 2004a). Whilst not used routinely in clinical practice, there is some suggestion that it may be useful in the asthmatic population (Nuttall et al. 2019).

Evidence for use of LCI, S_{cond} and S_{acin} in preterm-born children is limited and conflicting. Several studies showed little or no difference between preterm-born infants and term-born controls (Lum et al. 2011; Yammine et al. 2016). However, a more recent observational study of school-aged extremely preterm-born children showed that LCI was statistically higher in preterm-born children with CLD compared to term-born controls. There was no evidence of difference in LCI between preterm-born children without CLD and term-born children. (Sørensen et al. 2018). It is unclear why the available evidence does not conclusively demonstrate LCI is as useful in the preterm-born population but might suggest that the airway obstruction is fixed and does not allow gas to permeate distally.

1.3.5 Impulse Oscillometry

Impulse Oscillometry, also called forced oscillation technique (FOT), uses superimposed oscillations to measure changes in pressure and flow to assess respiratory mechanics (Skylogianni et al. 2016). In addition to evaluating peripheral airways, there is evidence of correlation between the FOT and spirometry (Broström et al. 2010) which enables assessment of respiratory mechanics in children who are unable to perform spirometry. Despite these benefits, currently there is a lack of validated reference ranges and a recognised inability of this technique to discriminate between obstructive and restrictive lung function deficits (Oostveen et al. 2003).

1.3.6 Electromagnetic inductance plethysmography (EIP)

EIP is a non-invasive test which does not depend on cognitive or physical ability. It uses voltage change within a generated electromagnetic field which are proportional to thoracic and/or abdominal movement to assesses tidal breathing (Williams et al. 2011). Current equipment requires the infant to wear a jacket or vest which creates the electromagnetic field and additional sensor equipment that measures the voltage change during breathing. Several studies have demonstrated their usefulness in evaluating tidal breathing in infants (Pickerd et al. 2013; Bentsen et al. 2016). However, its use continues to be limited due to the need for highly specialist equipment and personnel.

EIP provides potential opportunities to evaluate lung function in infants, children, and adults. Future use of these techniques in longitudinal studies may help establish predictive lung function measures which will assist in improved diagnosis and management of preterm-born children and adults.

1.3.7 Fractional exhaled nitric oxide (FeNO)

Nitric oxide (NO) is a signalling molecule that has several functions; neurotransmission, relaxation of smooth muscle, modulation of inflammation and host defence (Robbins and Grisham 1997). In the lung, NO acts on bronchial smooth muscle tone, pulmonary vasculature, and is involved in inflammation and mucous production (Antosova et al. 2017).

NO is synthesised by a family of nitric oxide synthase (NOS) which metabolise L-arginine into NO and L-citrulline. There are three isoforms of NOS; the calcium dependent neuronal (NOS I) and endothelial (NOS III), and calcium independent inducible (iNOS) which is controlled by proinflammatory stimuli. NOS I and NOS III are produced in the alveolar and bronchial epithelium; endothelial cells of the pulmonary vasculature; and specific neurons. iNOS is responsible for large increases of NO production (up to 20 times) in response to a pro-inflammatory stimulus.

Atopic asthma is associated with elevated levels of T-helper cell type-2 cells (Th_2) and eosinophilia. Secretion of cytokines IL-4, -5 and -13 drive eosinophil-linked inflammation, increased iNOS and NO production, leading to airway obstruction, hyperreactivity and remodelling (Robinson et al. 2017; Duong-Quy 2019).

Eosinophilia is assessed with BAL, or induced sputum. However, this is a challenging test to perform and assess, especially in children. Alternative tests including serum IgE and eosinophils have been found to have moderate diagnostic value (Korevaar et al. 2015).

There is evidence of upregulation of iNOS and elevated levels of FeNO in those with atopic asthma (Alving and Malinovschi 2010). Thus, FeNO measurement can be used as a biomarker for Th_2 driven inflammation. FeNO is a non-invasive, point of care test, which has good correlation with serum eosinophils, IgE, positive aero allergen skin tests and oesophageal eosinophil numbers (Strunk et al. 2003; Nakwan et al. 2022). Studies in children have demonstrated that FeNO is a useful test to help diagnose asthma, particularly allergic asthma (Ciprandi et al. 2013; Murray et al. 2017). It is useful to monitor asthmatic exacerbations (Petsky et al. 2018).

Despite its use in both adult and paediatric settings to aid the diagnosis and management of asthma (NICE 2017a; Khatri et al. 2021), the interpretation of FeNO levels and cut-off points for diagnosis continue to be based on limited evidence (Dweik 2011). Testing is also affected to the ingestion of medications (corticosteroids and leukotriene receptor agonists) and nitrate containing foods, alongside smoking and rhinovirus infections which can alter FeNO

measurements (Bjermer et al. 2014). Nevertheless, it is useful in monitoring response to treatment in asthma.

1.4 Lung function testing in infancy and early childhood

Lung function testing during infancy presents unique challenges. Raised-volume rapid thoracoabdominal compression and body plethysmography are tests which can be used to assess lung function in younger children who lack the ability to perform spirometry (Rosenfeld et al. 2013). However, these techniques are largely used in the research setting due to the high levels of expertise required, access to expensive equipment and use of sedation (Peterson-Carmichael et al. 2014). Thus, a greater number of studies focus on respiratory symptoms and healthcare utilisation compared to formal lung function during infancy and early childhood.

1.5 Rationale for choice of technique for assessing lung function

Spirometry enables the collection of accurate and objective lung function measurements before and after inhaled salbutamol. The measurement of FeNO enables greater assessment of the role of Th2 driven inflammation in those with lung function deficits. Spirometry, reversibility testing and FeNO are relatively easy to perform, cost effective and portable. Thus, they are the most accessible techniques for lung function testing in a high number of school-aged children in a community setting.

The collection of parent/child reported information in relation to historical and current respiratory symptoms and treatments, especially inhaled medication, further compliment lung function testing data.

1.6 Respiratory sequelae to preterm birth

Various lung function testing techniques have been used to report the respiratory sequelae during infancy, childhood and adulthood, after preterm birth. This section discusses the current evidence related to the respiratory sequelae after preterm birth.

1.6.1 Respiratory symptoms and health care utilisation

Preterm-born infants and children report greater respiratory symptoms and health care utilisation. Whilst these peak during infancy, for some, respiratory symptoms often continue to be experienced into adulthood.

In a large meta-analysis, Been et al demonstrated an association between preterm birth and wheezing which persisted beyond 5 years of age - suggesting potential life-long impact on respiratory function in this group. They also describe a decrease in the incidence of wheezing disorders of 6% for each extra week gestation in-utero (Figure 1-7) (Been et al. 2014).

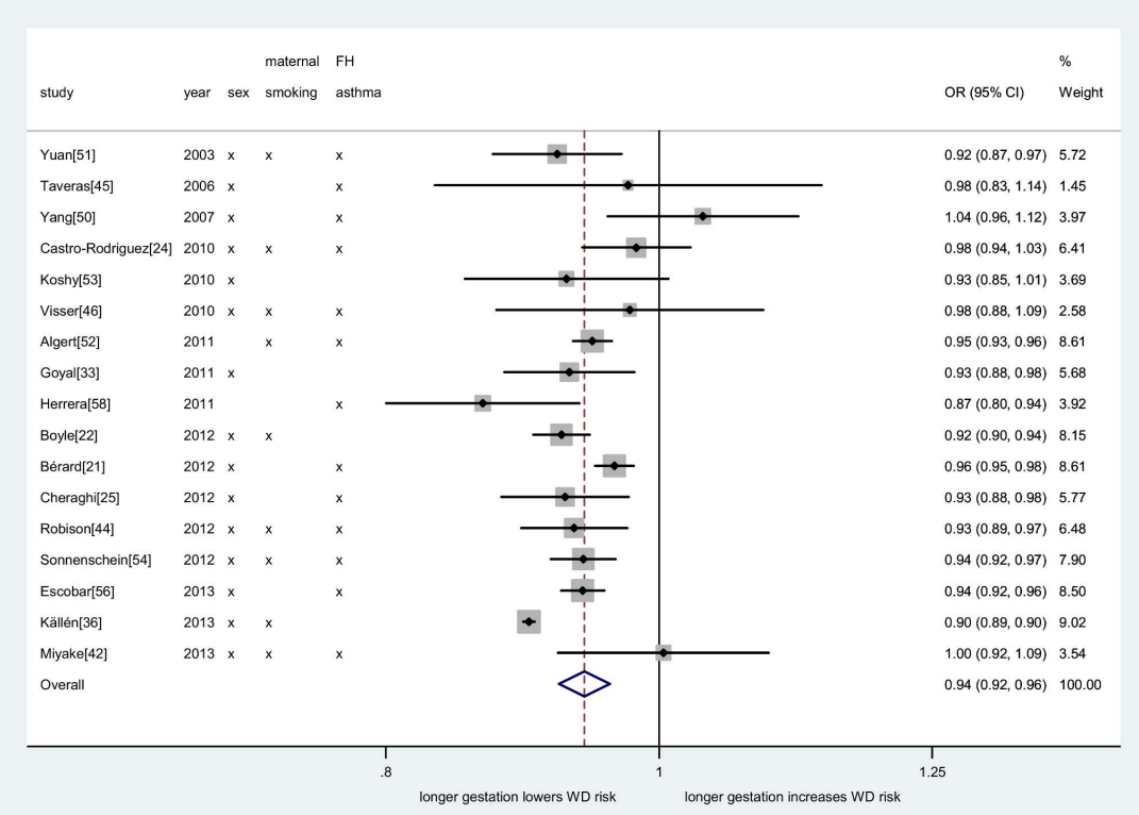


Figure 1-7 Forest plot showing the impact of decrease in gestation at birth on childhood wheezing.

The effect estimate is OR 0.94 (95% CI 0.92 to 0.96, p<0.01). Thus, 6% decrease in incidence for each week increase in gestation at birth. FH family history, WD wheezing disorder. (Been et al. 2014) Reproduced with permission under Creative Commons Attribution (CC BY) licence.

In a large cross-sectional population-based questionnaire, Edwards et al demonstrated an increased risk of wheeze-ever in preterm-born children who were both under 5 years of age and over 5 years of age. In both groups, the risk of wheeze increased with decreasing gestation at birth and was also associated with diagnosis of CLD in infancy. Levels of inhaler use were also higher in those with CLD. Reported wheeze-ever was independent of family history of atopy, suggesting that symptoms are related to prematurity and may not be due to atopy as often occurs in asthma (Edwards et al. 2016). This concurs with Broström et al who noted that - despite asthma like symptoms in those with CLD - there was no correlation with atopy (Broström et al. 2010).

Several studies demonstrate preterm-born children have greater respiratory symptoms, with those with CLD being most affected.

A large UK based longitudinal study showed that infants born at ≤25 weeks' gestation had higher levels of poor respiratory health than controls, with those with CLD having higher level of respiratory symptoms and medication use than those without CLD at both 30 months and 6 years age (Hennessy et al. 2008). At age 11 years, children in this study showed higher risk for diagnosis of asthma, use of asthma medication and nocturnal cough or exercise-induced wheeze in the prior 12 months, compared to term-born controls (Fawke et al. 2010).

The use of Inhaled bronchodilators in neonates born at <28 weeks' gestation participating in the American based Prematurity and Respiratory Outcomes Program (PROP) cohort study increased during the first year of life from 13% in the first 3 months to 31% by 12 months age. Use was higher in those with CLD (Ryan et al. 2019).

Whilst some preterm-born children receive inhaled medications, there is some evidence that preterm-born children have lower levels of treatment compared with asthma diagnosis (Hennessy et al. 2008). This inconsistency may reflect the dearth of evidence in relation to the effectiveness of inhaled medications on lung function in children born preterm (Kotecha et al. 2015). Both the European Respiratory Society (ERS) and American Thoracic Society (ATS) recognise the limited evidence base of optimal respiratory medication use in preterm-born children and adolescents. Recent recommendations from both societies suggest that inhaled bronchodilator and inhaled corticosteroids should be trialled in those with respiratory symptoms with close monitoring for response and to continue or discontinue treatment after re-evaluation (Duijts et al. 2020; Cristea et al. 2021).

Preterm-born adults - born in the pre-surfactant era - continue to report higher rates of asthma, cough and wheeze compared to term-born controls (Narang et al. 2008). Adults (16 – 25 years of age) born at <37 weeks' gestation showed that compared with those with a history of RDS, those with prior CLD were twice as likely to use respiratory medications and have a diagnosis of asthma (Landry et al. 2012). In a cohort of preterm-born adults who had moderate or severe CLD, 71% reported respiratory symptoms in the preceding 12 months (Wong et al. 2008).

Alongside increased respiratory symptoms, preterm-born children have greater health care use - particularly during infancy. A large proportion of admissions are related to respiratory illness - with those diagnosed with CLD in infancy requiring the greatest number of hospital admissions (Pramana et al. 2011; Berard et al. 2012). In a large population study of over 300,000 children, Paranjothy et al demonstrated that in the first year of life infants born at

39 weeks' gestation had an increased risk of hospital admission compared to infants born at >40 weeks' gestation. The risk of admission due to respiratory illness was inversely correlated with lower gestational age - with 41.5% of infants born at <33 weeks' gestation in the first year of life being admitted compared to 7.8% of those born at >40 weeks' (Paranjothy et al. 2013). This is consistent with the suggestion that maladaptation of the immune system occurs in preterm-born infants exposed to antenatal inflammation/infection (Kunzmann et al. 2013). Thus, leading to the inability to mount an appropriate response to postnatal infections leading to further lung injury. Indeed, preterm-born infants who sustain a lower respiratory tract infection in infancy have evidence of reduced lung function at 1 year of age (Drysdale et al. 2014). Whilst lung function testing in the youngest children is challenging, they have a greater incidence of respiratory symptoms and need for medical treatment than those born at term.

The inverse relationship between gestational age and respiratory wheeze and the increased reported incidence in those with CLD suggests that lung injury in the neonatal period has a lasting effect into childhood and early adulthood. Increased incidence of asthma diagnosis independent of atopy in this population suggests prematurity is responsible for a proportion of respiratory symptoms and the diagnostic label of asthma may be inappropriate. This, alongside the poor evidence in relation to inhaler use, requires further investigation.

1.6.2 Lung function in infancy and early childhood

Whilst observation of symptom occurrence and health care utilisation helps researchers to identify populations at risk, lung function testing helps to objectively assess the nature of respiratory function deficits and their response to treatment.

A study of 166 preterm-born infants demonstrated altered breathing patterns, with increased tidal volumes, lower respiratory rates, and decreased time to peak tidal expiratory flow/expiratory time ratio which corresponded with wheeze in the first year of life (Proietti et al. 2014). Whilst this is of interest, the overall predictive value of infant lung function testing is unclear. Thunqvist et al demonstrated decreased lung function in infants born at <30 weeks' gestation diagnosed with CLD. Sub-group analysis showed decreased compliance at 6 months, which improved by 18 months - suggesting ongoing lung development and remodelling of airways (Thunqvist et al. 2015).

1.6.3 Lung function in childhood

Greater capacity to perform more sophisticated lung function testing occurs with increasing age. Thus, a wide variety of lung function testing methods are available to assess childhood lung function in those born preterm. Despite this, a large amount of testing continues to be limited to the research setting, where there is access to both expertise and expensive equipment. This particularly applies to laboratory-based pulmonary function testing (PFT) which is viewed as the gold standard of testing. Several studies use a combination of different tests to enable a more comprehensive understanding of lung function in the preterm-born child. The use of computerised tomography (CT) scanning and - more recently - magnetic resonance imaging (MRI) scanning, including hyperpolarised xenon MRI scanning, have also been incorporated into some study protocols to assess structural lung development in preterm-born children.

Altered airway function in preterm-born children has been demonstrated in several studies using a variety of testing methods. Hennessy et al demonstrated lower peak expiratory flows (PEF) in preterm-born children at 6 years compared to term-born classmates, with those diagnosed with CLD having the lowest peak flows (Hennessy et al. 2008). Spirometry in the same cohort at aged 11 years was lower in the preterm-born group compared to term-born controls, with those with CLD having the greatest deficits (Fawke et al. 2010).

Kotecha et al's meta-analysis showed the mean %FEV_1 in preterm-born children with and without CLD is 8.7% lower than term-born controls (Figure 1-8). Variation in the studies included in their meta-analysis were most likely due to recruitment of different populations; different time periods (including pre-and post-surfactant), different gestational age, different ethnicities and therefore, results need to be interpreted with caution (Kotecha et al. 2013).

However, there remains a large body of studies demonstrating that preterm-born children have consistently lower spirometry, in particular, impaired forced expiratory volume in 1 second (FEV_1) compared to term-born controls (Kotecha et al. 2012b; Kotecha et al. 2013; Verheggen et al. 2016; Simpson et al. 2017).

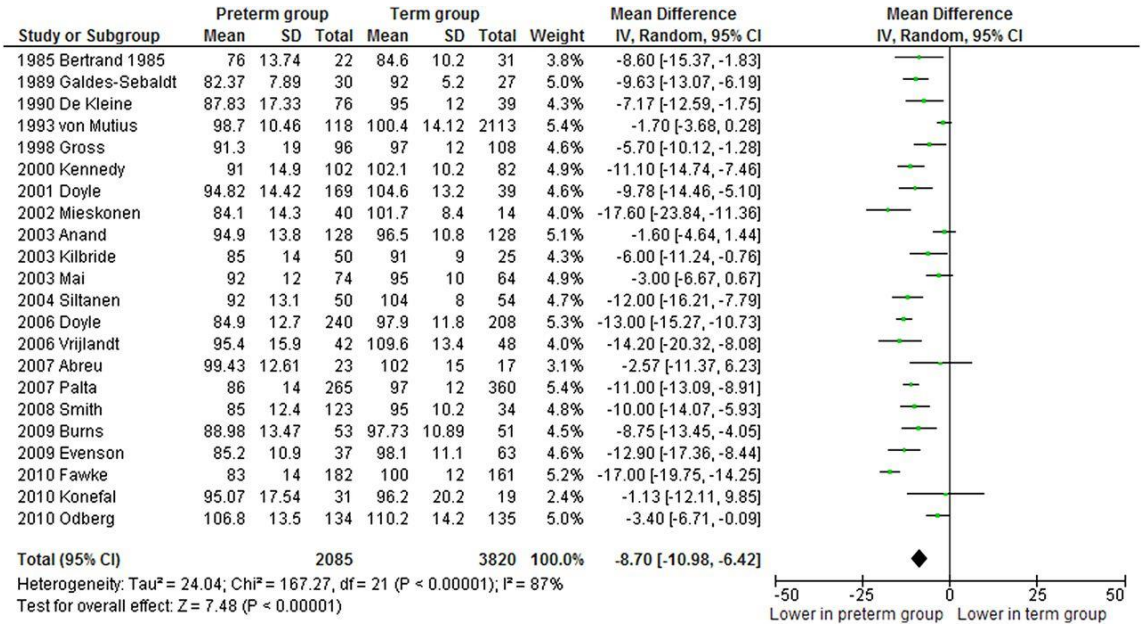


Figure 1-8 Forest plot showing pooled mean difference in percent predicted FEV_1 in preterm-born children compared to term-born controls.
Studies in analysis included preterm-born children with and without CLD (Kotecha et al. 2013) Reprinted with permission.

Body plethysmography and FOT have also been used to assess airways resistance. Studies employing these methods in the preterm-born population describe altered function of both central and peripheral airways resulting in altered airflow which was also observed on spirometry. By comparing preterm-born children (mean age 8 years) with and without CLD against term-born controls, Malmberg et al demonstrated elevated airways resistance by oscillometry in all preterm-born participants. Results were concordant with spirometry findings - which were lower in preterm-born children compared to term-born controls. Those with CLD were noted to have the lowest spirometry (Malmberg et al. 2000). Brostrom et al found more negative reactance in preterm-born children aged between 6 and 8 years with CLD with findings that are also concordant with FEV_1 on spirometry (Broström et al. 2010).

A small longitudinal study by Um-Bergström et al examined preterm-born children with and without CLD at 7 years and during adolescence (mean age 14.5 years). They reported elevated resistance on oscillometry in preterm-born children who had a history of severe CLD. They also described a decreasing trend in FEV_1/FVC associated with CLD suggesting increasing obstruction (Um-Bergström et al. 2017). Similarly, Lo et al demonstrate longitudinal decreases in the ratio between functional residual capacity on helium dilution and plethysmography (FRC_{he}:FRC_{pleth}) between 1 year and 11 - 14 years in preterm-born children, suggesting worsening small airways function which may lead to significant long-

term lung function deficits (Lo et al. 2018).

In addition to altered airway calibre, Choukroun et al describe increased RV and RV/TLC in preterm-born school-aged children suggestive of air trapping that is most often a consequence of obstructive lung disease (Choukroun et al. 2015). These findings concur with Cazzato et al who describe elevated levels of RV and RV/TLC in preterm-born children with a mean age of 8 years compared to term-born controls. These levels were greatest in preterm-born children who had CLD (Cazzato et al. 2013).

A higher incidence of bronchial hyper-responsiveness in preterm-born children - particularly those born extremely preterm (<28 weeks' gestation) - is well described (Kotecha et al. 2018). However, the mechanisms for this are unclear. Unlike asthmatic children, Kim et al describe how preterm-born pre-school aged children with a history of CLD have hyper-responsiveness to methacholine and not adenosine 5'monophosphate (AMP), suggesting airway smooth muscle hypertrophy as opposed to airway inflammation may be responsible for hyper-responsiveness (Kim et al. 2006). This further supports the hypothesis that maladaptation of the airways may contribute towards lung function deficits.

In addition to functional impairment, several studies have demonstrated structural alterations in children born preterm by using CT scanning. Aquino et al demonstrated abnormal decreases in density and air-trapping in preterm-born children with CLD (Aquino et al. 1999). A large study by Simpson et al demonstrated ongoing impaired lung function in all children born at ≤32 weeks' gestation compared to term-born controls, with preterm-born children with CLD having the largest deficits. Concurrent CT scans showed 92% of preterm-born children had structural abnormalities - these changes correlated with the findings of lung function testing and higher reported respiratory symptoms - with greater structural changes in those with evidence of obstructive disease (Simpson et al. 2017).

Preterm-born children have lung function deficits that can be tracked into childhood alongside changes in lung architecture. Children with a history of CLD in infancy have the greatest lung function deficits that are often described as obstructive in nature and likely to be a consequence of airway hypertrophy. However, several studies recruited children from the pre-surfactant era thus, limiting generalisability of findings to today's preterm-born children, or limited analysis to comparing preterm-born children with and without CLD. These limitations result in a lack of comprehensive understanding of the nature of lung function deficits identified in preterm-born children with and without CLD.

1.6.4 Lung function in adulthood

The impact of early life health on adult respiratory health has been well documented. In a large cohort study of men born with low birthweight, Barker et al demonstrated worse adult lung function and increased risk of death from chronic obstructive pulmonary disease (COPD) when compared to those born at normal birthweight (Barker et al. 1991).

Lung function trajectories progress in three phases: growth (lung function developing into early adulthood), plateau (early 20's) and decline (associated with lung ageing) (Gibbons et al. 2020). Adverse events during these phases can result in deviation away from the "normal" trajectory (Figure 1-9).

Preterm birth has a significant impact on the early growth phase often resulting in a lower lung function trajectory and development of lung disease earlier in adulthood. Stern et al demonstrated that - irrespective of gestation at birth - impaired infant lung function is correlated with lower lung function in adolescence and early adulthood. Thus, demonstrating the concept of lung function tracking through the life course. Additional follow-up of cohorts of children born with extremely low birth weight (ELBW) or extremely preterm have also suggested that the association between infant and childhood lung function continues into adulthood (Stern et al. 2007). Gibson et al demonstrated lower FEV_1 and FEV_1/FVC ratio in young adults (mean age 22.4 - 28.6 years) with a birthweight of <1500gms compared to those born weighing >2499gms. They also identified a correlation between FEV_1 in childhood and adulthood (Gibson et al. 2015).

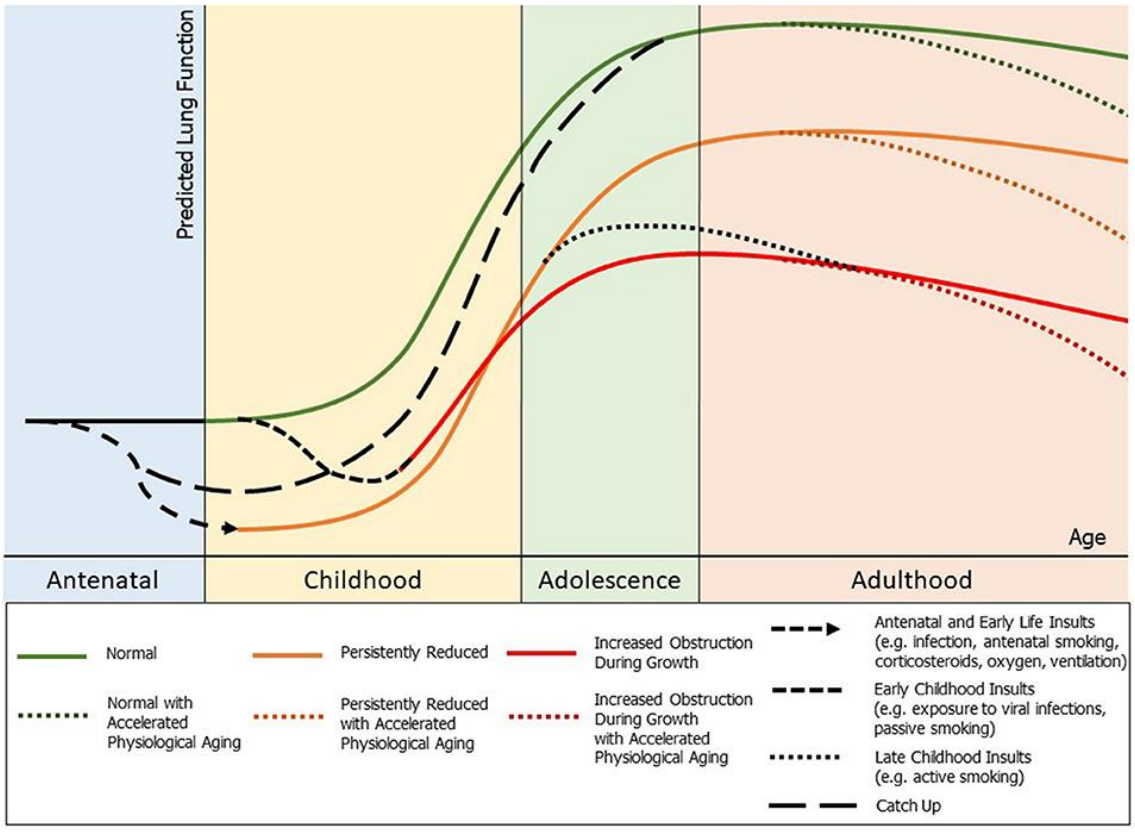


Figure 1-9 Diagrammatic representation of the impact that adverse events have on lung function trajectories.
Normal lung function trajectory (green), trajectory for those with persistent lung function deficits (orange) and trajectory with progressive obstruction (red). Additional impacts which may alter trajectories (smoking etc.) also identified. (Gibbons et al. 2020) Reproduced with permission under Creative Commons Attribution (CC BY) licence.

An accelerated decline in adult respiratory health is also associated with obstructive disease in childhood. Lange et al demonstrated that asthmatics with low %FEV_1 in early adulthood were three times more likely to develop COPD in midlife than those with a normal %FEV_1 (Lange et al. 2015). In the preterm-born population, obstructive lung function deficits in childhood and adolescence persist into early adulthood, with those with a history of CLD having the greatest deficits (Vollsaeter et al. 2013). Similarly, Doyle et al observed evidence of increasing airway obstruction (decreasing %FEV_1) in preterm-born children between aged 8 and 18 years. The decline was worst in those with a history of CLD and those currently smoking (Doyle et al. 2017).

Evidence of gas trapping, obstruction and bronchial hyper-responsiveness in a cohort of preterm-born young adults has also been described. A greater proportion of these preterm-

born adults had a positive BDR compared to term-born controls - with the greatest proportion of those with CLD having a response - suggesting that prematurity associated lung function deficits is obstructive rather than restrictive (Landry et al. 2016). Further comprehensive review of lung function and structure by Caskey et al demonstrated reduced diffusion capacity and greater obstructive disease in preterm-born adults than term-born controls (Caskey et al. 2016). In those with CLD, CT scans showed impaired pulmonary structure, suggesting lung function deficits may be due to altered/maladapted airway growth and development. Thus, preterm birth may contribute to initial lung function deficits, reduced lung function trajectories, accelerated decline in function and potential for earlier development of COPD.

Additional lifestyle factors and childhood respiratory illness (acute and chronic) play a role in the acceleration in the rate of decline in respiratory function with smoking, exposure to pollution, viral infections, genetic factors, and deprivation all being highlighted as having a potential role (Stocks et al. 2013; Gibbons et al. 2020; Bush 2021).

The benefits of exercise on long-term health outcomes are well documented. Evidence that children and adults born preterm have lower tolerance of exercise – possibly due to exercised induced bronchospasm (Joshi et al. 2013; Harris et al. 2020) - and lead more sedentary lifestyles (Lowe et al. 2016) has the potential to further compromise an individual's lung function trajectory.

In addition to addressing deprivation and reducing the risk of preterm birth, identification of strategies to improve lung function and targeted health promotion (exercise, smoking cessation) may help to maximise lung function trajectories and reduce additional insults that further reduce lung function

Current lung function studies involving preterm-born adults include those born in the pre-surfactant era, when different ventilation techniques and oxygen management strategies were also in use. Therefore, findings may not be transferrable to those born in the post-surfactant era. Whilst acknowledging the limitations of these adult based studies, the correlation between lung function in childhood and adulthood remains. Current childhood studies - including those studying those born in the post-surfactant era – continue to demonstrate ongoing lung function deficits in preterm-born children. Therefore, it is anticipated that future studies will demonstrate preterm birth continues to have a significant

impact on adult lung function.

Bolton et al identified the lack of perceived importance of early life events in adult respiratory medicine and suggest that the concept of lung function in infancy influencing lung trajectories and consequent adulthood respiratory disease is a vital consideration for future health care planning (Bolton et al. 2015).

1.7 Spirometry, reversibility, and inflammation in childhood

In view of the ongoing impact that preterm birth has upon lung function, it is reasonable to suggest that ongoing community lung function testing may help identify children and adults with lung function deficits.

Spirometry and reversibility testing are the most commonly used lung function tests used to evaluate lung function in both children and adults in health care settings and research studies. Used in most studies evaluating lung function of children born preterm, spirometry and reversibility testing are perfectly placed to inform large community-based research studies. As such, it has been used to assess the cohort in this thesis. Further discussion of current evidence specific to spirometry measures and FeNO in school-aged preterm-born children is presented in this section.

1.7.1 Spirometry in the preterm-born child

Interpretation of the evidence related to spirometry outcomes in preterm-born children is challenging due to several factors. Due to the limited population, results in some studies draw conclusions from small sample sizes resulting in an underpowered study. Some studies were undertaken prior to the routine neonatal installation of exogenous surfactant - limiting the application of conclusions to children born in the post-surfactant era. There is a high level of variability in study populations born at different gestational ages, with earlier studies selecting those born at lower gestations, or extremely low birthweight as opposed to more recent studies which aim to incorporate children born moderately preterm. Some studies used birthweight as a surrogate for gestation - raising the potential that IUGR may be an unaccounted factor influencing outcomes. Not all studies included term-born controls and a proportion have concentrated on defining the outcomes of children with CLD, preventing comprehensive understanding of the lung function deficits in the whole preterm-born population.

In studies focussing on CLD, the use of different diagnostic criteria makes comparison between cohorts challenging. In recognising these limitations, current available evidence demonstrates spirometry in preterm-born children is lower compared to term-born controls, with those with CLD having the greatest deficits of 18.92% (Figure 1-10) (Kotecha et al. 2013).

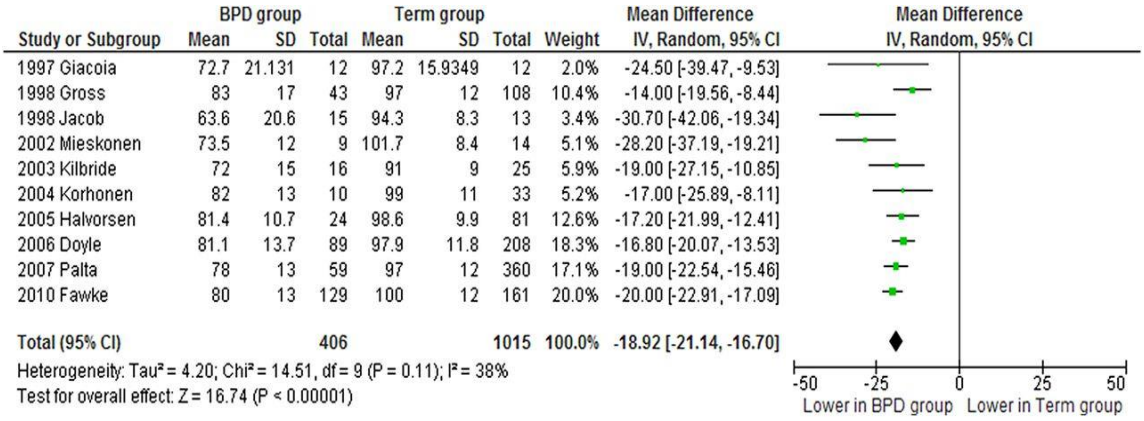


Figure 1-10 Forest plot showing pooled mean difference in percent predicted FEV_1 between preterm-born children with CLD and term-born controls *(Kotecha et al. 2013) Reprinted with permission.*

Three cohorts recruited by a team in Norway describe the lung function of children born ELBW and <28 weeks' gestation (extremely preterm - EPT). All cohorts included preterm- and term-born children. Two cohorts included children/adolescents born in the pre- surfactant era and one cohort included children born in the post-surfactant era. Severity of CLD was defined as mild (supplemental oxygen at ≥28 days of age) and moderate/severe (supplemental oxygen at ≥36 weeks' PMA).

The earliest cohort consisted of 46 preterm-born adolescents and 46 age matched controls born 1982 – 85 (1980's cohort). The preterm-born group had significantly lower PEF (no CLD -3.5%, mild CLD -12.3%, moderate/severe CLD -16.3%) and FEV_1 (no CLD -6.3%, mild -12%, moderate/severe -20.3%) than term-born controls. The association between low FEV_1 and increasing severity of CLD led the authors to conclude that CLD was a significant predictor of lung function deficits in the 1980's cohort (Halvorsen et al. 2004).

Comparison of the 1980's cohort with a second cohort of 35 preterm-born children and 35 age matched term-born controls born in 1991 - 1992 (early 1990's cohort) showed comparable lung function deficits that were similarly associated with severity of neonatal

lung disease/CLD (Halvorsen et al. 2006).These findings demonstrate that preterm-born children from the pre-surfactant era diagnosed with CLD are at highest risk for lung function deficits in childhood.

The third cohort of children - born 1999-2000 (late 1990's cohort) and born in the post-surfactant era - had better lung function than the early 1990's cohort (Vollsaeter et al. 2015). This suggests that surfactant may have a role in improving respiratory outcomes in the preterm-born population. Despite this improved lung function, spirometry measures in this preterm-born cohort continued to be lower than term-born controls. Unlike the earlier cohorts, lung function deficits in the late 1990's cohort were not associated with CLD. Similar findings are described by Cazzato et al who demonstrated that, whilst school-aged children born <32 weeks' gestation have lower FEV_1, FVC and $FEF_{25-75\%}$ compared to controls, there were no differences between preterm-born participants with and without CLD (Cazzato et al. 2013). Whilst it could be suggested that these findings may reflect the changing pathology of CLD in the post-surfactant era, it is possible that the small sample size in both studies may have resulted in an inability to detect a difference between the group with CLD and the group without CLD.

A study of younger children aged 4-8 years also found that children born <32 weeks' gestation had lower spirometry compared to term-born controls, but no significant differences between preterm-born children with and without CLD (Verheggen et al. 2016). Follow-up of these children aged 9 - 11 years showed spirometry deficits between preterm- and term-born controls continued and those with CLD had greatest deficits (Simpson et al. 2017) - suggesting that the impact of CLD may not be identifiable on spirometry until mid-childhood.

Fortuna et al also demonstrated that preterm-born children born EPT and ELBW had lower spirometry values compared to term-born children (Fortuna et al. 2016). However, contrary to Cazzato et al and Vollsaeter et al, they found those with CLD had the lowest spirometry. They also demonstrated that between the ages of 8 and 12 years, spirometry in the preterm-born group with CLD declined, leading the authors to suggest that preterm-born children with CLD exhibit lower spirometry with ongoing decline in lung function during mid-childhood. This decline was not seen in the preterm-born group without CLD (Fortuna et al. 2016).

A study of ELBW preterm-born Swedish children aged 6-8 years also describe children with CLD having lower spirometry than those without CLD (Broström et al. 2010). A higher proportion of the CLD group had a %FEV_1 below normal limits (50%) compared to the non-CLD group (17%). Whilst the FVC in the non-CLD group was normal, it was reduced in the CLD group. Subsequent follow up of this cohort in adolescence showed longitudinal decrease in FEV_1/FVC z-scores that correlated to severity of CLD, suggesting that those with CLD are at potential increased risk of worsening airway obstruction (Um-Bergström et al. 2017).

The Victoria Collaborative Infant Study Group recruited ELBW and EPT children born in two different time periods - 1992 (early 90's cohort) and 1997 (late 90's cohort). Both cohorts recruited children born in the post-surfactant era and term-born controls. The early 90's cohort was tested at age 8 and 18 years. At 8 years, there was evidence of lung function deficits in the preterm-born population compared to term-born controls with 19.7% of preterm-born children having a %FEV_1 below 75% compared with 2.4% of term-born controls. A greater proportion of the preterm-born group also had evidence of obstructive disease with an FEV_1/FVC ratio <75% predicted value. Sub-group analysis showed that the preterm-born group with CLD had the greatest proportion of children with low FEV_1 (Doyle et al. 2006). Follow-up at 18 years showed the preterm-born group continued to have lower spirometry values compared to term-born controls and that those with CLD had the greatest deficits (Doyle et al. 2017). At 8 years of age, children in both the early 90's and late 90's cohorts had significant lung function impairments compared to term-born controls, with those with CLD having the lowest spirometry (Hacking et al. 2013).

The EPIcure study demonstrated that compared to 9% of term-born controls, 32% preterm-born without CLD and 66% preterm-born children with CLD had abnormally low spirometry readings (Fawke et al. 2010). Limiting recruitment to those born at ≤25 weeks' gestation in the post-surfactant era, focussed on those most likely to have profound lung function deficits. However, more recent evidence has suggested that children and adolescents born at later gestations may also be a risk for lung function deficits.

Spirometry measures in children (aged 8-9 years) and adolescents (aged 14-17 years) who were born at later gestational ages were assessed in a large UK cohort. Findings from this study demonstrated that at 8-9 years of age those born at 35 - 36 weeks' gestation had spirometry comparable to term-born controls. However, those born at 33-34 weeks' gestation had reduced spirometry readings which were comparable to those born at 25-32 weeks' gestation. Observed decrements in FEV_1 and FVC, but not FEV_1/FVC and $FEF_{25-75\%}$ in

those born 33-34 weeks' gestation improved by adolescence(Kotecha et al. 2012a). The reason for this remains unexplained, but growth and maturation may explain these improvements. Despite the evidence that those born 33-34 weeks' gestation show improvements in spirometry measures as their age increases, it is reasonable to suggest that including those born at ≤34 weeks' gestation in the current study should provide a more comprehensive understanding of the impact of prematurity on lung function in childhood, adolescence, and adulthood.

These studies suggest that - despite the routine use of maternal antenatal corticosteroids and neonatal installation of exogenous surfactant - preterm-born children continue to have deficits in spirometry compared to term-born controls. Whilst two studies in the post-surfactant era did not find differences between those with and without CLD, many studies continue to identify CLD as a risk factor for lung function deficits in childhood. Indeed, several studies suggest that children with previous CLD have lower spirometry that is obstructive in nature and continues to decline with age. However, these studies also identify a group of preterm-born children who have ongoing lung function deficits which is not associated with CLD. To date, in-depth investigation into the nature and potential causes of lung function deficits in this group of children born has not been undertaken.

1.7.2 BDR and the preterm-born population

Several studies have included reversibility testing to assess respiratory function in the preterm-born population. Like the information related to spirometry in this population, evidence is limited by small sample size, recruitment of children at different gestations, a lack of term-born controls and primary focus on those with CLD. There are also variations in the selection of type, dose, and delivery method of bronchodilator used - with salbutamol, isoproterenol and terbutaline all used. Despite these differences, most studies demonstrate an improvement in FEV_1 in preterm-born children following inhalation of a single dose of bronchodilator, with those with CLD with the largest FEV_1 deficits having the greatest response (Kotecha et al. 2015).

Whilst findings by Kotecha et al are consistent with evidence suggesting that lung function deficits in those with CLD is obstructive in nature, several studies demonstrate that reversibility is not observed in all these children (Filippone et al. 2003; Vom Hove et al. 2014; Kotecha et al. 2015; Fortuna et al. 2016), nor is it exclusive to those with CLD; with up to 25% preterm-born children without CLD having deficits on spirometry and demonstrating a

positive BDR (Vom Hove et al. 2014). These findings suggest that further analysis of the extent and nature of spirometry deficits and response to bronchodilator in the whole preterm-born population is required.

1.7.3 FeNO in the preterm-born population

Whilst there is correlation between the development of CLD and inflammatory responses in preterm-born infants (Kotecha et al. 2003; Chakraborty et al. 2013; Balany and Bhandari 2015), there is no evidence directly linking CLD with Th_2 driven eosinophilic inflammation.

Assessment of FeNO levels in the first week of life in preterm-born infants showed no correlation between CLD and levels of FeNO (Williams et al. 2007). Indeed, it has been suggested that, compared to term-born controls, spontaneously breathing preterm-born infants in the first two days of life and school-aged children with CLD have lower levels of FeNO (Condò et al. 2003; Baraldi et al. 2005). Furthermore, Nordlund et al showed that - despite having lower spirometry measures - preterm-born children with CLD had significantly lower FeNO compared to term-born children with atopic asthma - suggesting the underlying mechanism for lung function deficits in preterm-born children differs to those with asthma (Nordlund et al. 2017).

A recent SR and meta-analysis concluded that preterm-born subjects - irrespective of CLD status - had FeNO comparable to term-born controls, suggesting Th2 driven eosinophilic inflammation is unlikely to explain lung function deficits described in the preterm-born population (Course et al. 2019). The inclusion of studies with children and adults born at <37 weeks' gestation born in the both the pre- and post-surfactant era may limit the generalisability of findings to preterm-born children with ongoing lung function deficits.

Extremely preterm-born school-aged children with lung function deficits born in the post-surfactant era have been found to have FeNO levels similar to those found in term-born controls, irrespective of CLD status (Cazzato et al. 2013; Fortuna et al. 2016). Whilst there is evidence to demonstrate preterm-born children with spirometry deficits have normal levels of FeNO, to date there has been no comprehensive analysis of the FeNO levels in preterm-born children who demonstrate reversible lung function deficits. There is a need to review FeNO levels in the preterm-born population accounting for current lung function measures, including reversibility.

1.8 The RHiNO Study

This thesis comprises information collected as part of a larger study - the RHiNO study - a three-part Medical Research Council sponsored study which aimed to recruit a large cohort of preterm- and term-born school-aged children. The three parts are described below:

Part 1. Screening: Anthropometric and cardiovascular assessments, collection of demographic data and information related to respiratory symptoms, spirometry, reversibility testing and FeNO analysis in a community environment.

Part 2. RCT: Formal pulmonary function testing in a hospital setting of both preterm- and term-born children. Testing included spirometry, reversibility testing, FOT, body plethysmography, helium dilution, FeNO, cardiovascular assessment, exercise tolerance testing, allergy testing, collection of exhaled breath condensate and induced sputum. Randomisation of preterm-born children with a $\%FEV_1$ ≤85% to three months of different inhaled therapies with subsequent re-testing to evaluate their impact was also undertaken.

Part 3. Imaging: Hyperpolarised MRI to assess the lung architecture.

As one of the largest trials of its kind, the RHiNO study incorporated comprehensive testing in both preterm- and term-born children. Unlike previous studies who enrolled children born at lower gestations, the RHiNO study chose to enroll children born at ≤34 weeks' gestation. This choice reflects evidence suggesting that children born at 33-34 weeks' gestation have lung function deficits on spirometry comparable to those born at 25-32 weeks' gestation alongside evidence describing respiratory symptoms in those born in the moderately preterm-born population (Kotecha et al. 2012a; Edwards et al. 2015).

With suitably powered results it will provide high-quality data detailing the impact that preterm birth has on lung function, identify different respiratory phenotypes with a strong focus on the response to different inhaled therapies, and whether there is any associated evidence of altered lung structure.

1.9 Summary

Advances in health care provision have improved the outcomes for preterm-born children leading to many infants born at the edge of viability surviving. Concurrent emergence of a

new form of CLD in this group - largely associated with abnormal structural development - presents new challenges for health care providers. It is of concern that, as survival rates improve, a greater number of preterm-born children will not attain their maximal lung function trajectory and subsequently experience poor respiratory health and potentially develop COPD earlier in life. Greater understanding of the nature of lung disease and optimal treatment options are vital to ensure adequate healthcare planning and development of management strategies to improve long-term lung function outcomes.

Longitudinal studies continue to suggest that lung function in adulthood can be influenced by fetal and neonatal injury. Whilst lung function deficits in preterm-born children have been described, analysis has often been limited to those born EPT or ELBW and comparisons are often restricted to preterm-born children with and without CLD, with only a small number of studies referencing against a term-born population. These studies are also small and therefore have limited power.

In the post-surfactant era, there continues to be evidence linking CLD with poor respiratory outcomes. However, with evidence that some preterm-born children without CLD also have lung function deficits, and emerging criticism of the predictive value of CLD (Kotecha et al. 2013; Corwin et al. 2018), there is a need to re-evaluate lung function in a wider preterm-born population. Furthermore, whilst there is some suggestion that lung function deficits in the preterm-born population is obstructive in nature, this evidence is limited and often only described in those with CLD. There is a need to accurately describe lung function in a large preterm-born population - irrespective of previous diagnosis of CLD - with a focus on different respiratory phenotypes and whether associated lung function deficits are obstructive or non-obstructive in nature.

Although preterm-born children have similar levels of atopy as term-born controls, they continue to have higher levels of asthma diagnosis. Despite the higher incidence of asthma diagnosis, there continues to be inconsistent prescription and use of inhaled medication in this group. This is a likely consequence of the paucity of evidence clarifying the optimal use of bronchodilators in these children. In addition to describing the response to inhaled bronchodilator within the whole preterm-born population, greater understanding of different respiratory phenotypes and their response to bronchodilators may assist in greater targeting of therapies for this vulnerable group of children. Furthermore, there is a need for clarification of whether measurement of FeNO is associated with lung function deficits in the preterm-born population and, if so, whether it is associated with any respiratory phenotypes.

1.10 Hypothesis and Aims of the study

Part 1 of the RHiNO study was designed to screen and identify children who could participate in the RCT (part 2). I formulated formal questions to address specific hypotheses and aims using data collected during the community-based assessments as follows:

Hypotheses:

1) Preterm-born children have greater lung function deficits than term-born children

2) a. Preterm-born children with a history of CLD in infancy have greater incidence of lung function deficits compared to term- and preterm-born children with no history of CLD.

   b. CLD is the optimal predictor of lung function deficits in preterm-born school-aged children.

3) a. Lung function deficits in preterm-born children can be classified as either obstructive or non-obstructive in nature.

   b. Preterm-born children with obstructive lung function deficits have a greater response to a single dose of bronchodilator than those with non-obstructive lung function deficits.

4) Lung function deficits in preterm-born children is independent of Th2 driven eosinophilic asthma.

Specific aims:

- To describe lung function in a population of children aged 7 - 12 years of age born at ≤34 weeks' gestation and compare results to term-born controls of equivalent age.
- To explore early life factors associated with lung function deficits in childhood.
- To accurately identify lung function deficits in preterm-born children and classify the deficits into obstructive or non-obstructive airway disease using a combination of FEV_1 and FEV_1/FVC ratios.
- To identify the reversibility of airflow limitation following 400mcg salbutamol in preterm-born children with lung function deficits classified as either obstructive or non-obstructive.

2 Methods

This chapter describes the recruitment strategy, study set-up and testing methods to address the specific aims and hypothesis outlined in Chapter One.

2.1 Recruitment of participants

To ensure equitable and appropriate recruitment, clear inclusion and exclusion criteria were specified:

Inclusion criteria

- Children aged 7 - 12 years born at ≤34 weeks' gestation comprised the preterm-born group
- Children aged 7-12 years born at ≥37 weeks' gestation comprised the term-born control group
- Living in South Wales, so accessible to home visits
- Responded to a postal questionnaire and willing to participate in a community visit
- Free of respiratory illness in the 3 weeks prior to testing

Children with a history of respiratory illness in the preceding 3 weeks had their visit deferred until they had been free of respiratory symptoms for at least three weeks.

Exclusion criteria

- Congenital malformations
- Severe cardiopulmonary defects
- Neuromuscular disease, which would limit the ability to perform the testing
- Severe neurodevelopmental impairment which - in the opinion of the investigator - would limit the child's ability to perform the testing.

2.1.1 Recruitment sources

The Respiratory and Neurological Outcomes in Preterm Children (RANOPs) study was a cross-sectional survey of preterm-born (<37 weeks' gestation) children born in Wales in 2003, 2005, 2007, 2009, 2010 and 2011 together with age-matched term-born controls (>37 weeks' gestation). Over 26,000 respiratory questionnaires were mailed to these children;

Children aged under 5 years completed a Liverpool Respiratory Symptom Questionnaire (Powell et al. 2002) and those 5-10 years completed an adapted ISAAC respiratory questionnaire (Asher et al. 1995; Joshi et al. 2013). 4,200 preterm- and 2,800 term-born children returned their completed respiratory questionnaire and agreed to future participation in similar research (Edwards et al. 2015).

Initial recruitment of both preterm- and term-born children focused on the children who had participated in the RANOPs study. To further increase the potential recruitment pool, children who had previously been invited to participate in the RANOPs study but did not respond were also invited to participate (after ethics approval). To enable home visits, the geographical area for recruitment was limited to South Wales only. Additional exclusions due to gestation at birth being >34 or ≤37 weeks' gestation and being over 12 years old at the time of recruitment inevitably resulted in a significant proportion of the RANOP's cohort being excluded.

Potential recruits from the previous RANOPs cohort (responders and non-responders) were sent a modified validated ISAAC respiratory questionnaire and an invitation letter to participate in a community visit. Information leaflets for both parent and child were enclosed with the questionnaire. Examples of parent and child information leaflets for the preterm- and term -born recruitment groups can be seen in Appendix 2 and 3.

An additional recruitment group of preterm-born children (new cohort) born in 2004, 2006 and 2008 who had not previously been invited to participate in the RANOPs study were also identified by the NHS Welsh Informatics System (NWIS). This process required additional ethics approval and the identification of a principal investigator (PI) at each of the four hospitals in South Wales which may be a source of potential recruits. Specific invitation letters which contained a tick box system for expressing interest or declining participation in the study were formulated and sent on behalf of each PI. Parent and child information leaflets about the study accompanied the letter. The ISAAC questionnaire was completed and reviewed during the community visit in those who agreed to participate.

NWIS provided the most up-to-date information for all contacts including home address and any relevant medical history. This information also highlighted children who had died to avoid the team contacting families inappropriately. This information was regularly updated.

To improve the response rate, two subsequent reminders were sent to non-responders at approximately 6 weeks and 12 weeks after the initial mailing. On receipt of a

questionnaire/letter indicating that the child and family were agreeable to participating in a community visit, using the preferred method of contact indicated by the family, a convenient date and time for the visit was arranged. In total, 3,601 preterm-born (2,197 RANOP's and 1,404 new cohort) and 1,180 term-born children received invites to participate. Table 2-1 details preterm-born and term-born mailing numbers by year of birth.

Year of birth	Preterm-born	Term-born
2004	163	0
2005	314	255
2006	584	0
2007	564	366
2008	655	0
2009	517	324
2010	511	238
2011	293	0
Total	**3601**	**1183**

Table 2-1 Number of preterm- and term-born children sent participation invites by year of birth.

2.2 Setting-up the community visit

The recruitment process was designed to encourage as many children and their families as possible to participate in the study whilst ensuring that the team could organise a community visit within as short a time as possible. Invitations were sent out in white envelopes with RHiNO branding labels in age-based mailing groups. Pre-paid envelopes were also included in the mailing pack to increase the number of returned questionnaires. Continual evaluation of the return rate of questionnaires, alongside the number of visits arranged and completed were undertaken on a weekly basis with adaptations to further invite mailings made accordingly.

All children and families willing to participate in the study were offered the choice of a visit at the Children and Young People's Research Unit (CYARU) at the University Hospital of Wales (UHW), or, at the child's home. Whilst acknowledging that providing the option of a community visit may improve recruitment, the nature of a community visit presented two areas of concern that needed to be addresses during the setting-up phase: lone working and administration of medication without a prescription.

2.2.1 Lone working

UK legislation requires the employer to ensure that the welfare of employees is considered,

with work associated risks assessed and provision of appropriate equipment and training to help reduce any identified risk (Health and Safety at Work Act 1974; The Management of Health and Safety at Work (Regulations) 1991). A risk assessment of the intended process for a lone worker performing a home visit was undertaken to ensure that potential hazards were considered, and appropriate actions taken to mitigate or reduce risk. Figure 2-1 shows the five-stage risk assessment process that was undertaken in relation to the home visits.

Lone working is an area of work that is specifically highlighted as presenting increased risk of harm to employees, with the risk of injury due to physical assault being 9% higher in lone working healthcare staff compared to non-lone workers (NHSProtect 2015). Unlike conventional heath care settings, the study team did not have access to information relevant to the child and family background to enable assessment of the potential risk of violence or abuse to lone working team members. There were additional risks associated with driving long distances in various weather conditions, often in the early evening. To reduce these risks, several different interventions, including the development of a unique standard operating procedure (SOP) were implemented (Appendix 4).

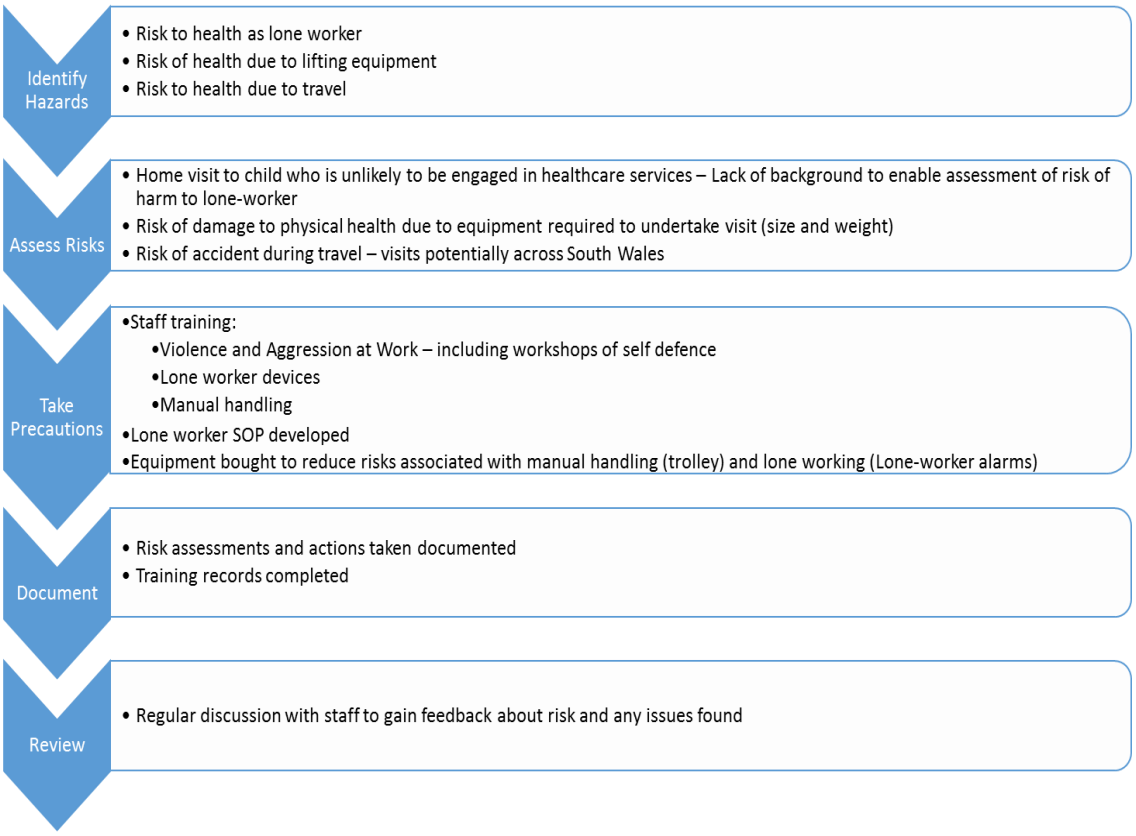


Figure 2-1 Five stage risk assessment process for lone worker home visits for the study.

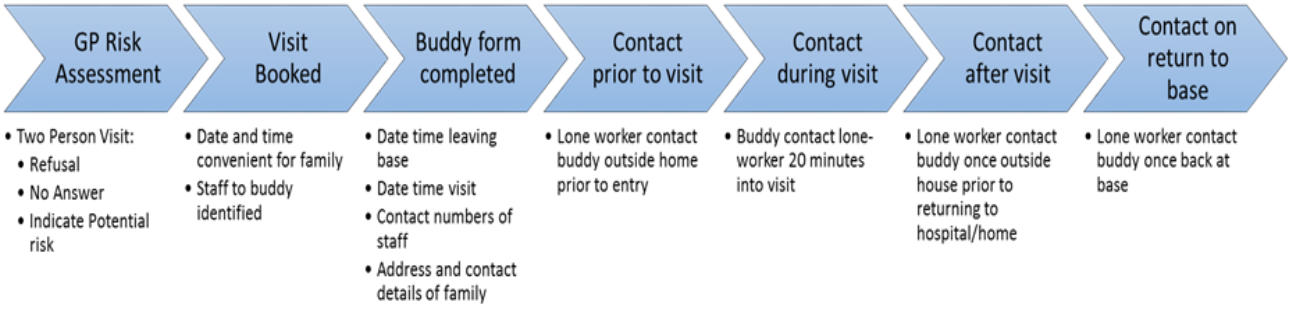


Figure 2-2 Key components of the lone worker SOP.
SOP specifically developed to enable a risk assessment of the family home prior to the visit, alongside providing a buddy system to support during the visit to ensure safety of lone workers undertaking a lone home visit as part of the study.

In addition to each staff member receiving the highest level of violence and aggression training, they were also provided with lone worker devices. Whilst both the training and lone worker devices provide ways of enabling lone workers to react and manage any potential incidents, I felt there was a need to develop a system of proactively assessing the likelihood of an adverse event occurring and ensure that each lone worker had safely returned to base post-visit.

The UHW has an information sharing agreement with the Local Medical Committee related to potential risk to healthcare workers (such as violent markers on medical notes). As such, we contacted GP practices asking them to identify any potential risks. Whilst most risk assessments were completed and indicated no known risk to those preparing to undertake a lone home visit, on occasions when the GP indicated a potential issue or did not complete the risk assessment form, either a second person attended the visit, or the family were asked to attend the hospital. Further risk reduction was provided by using a buddy system whereby the lone worker would have contact with an identified buddy before, during and after each visit and on return to base. Whilst there was limited risk reduction associated with travelling, the buddy system ensured that the lone worker had returned to base safely**.** Figure 2-2 outlines the key components of the SOP.

2.2.2 Administration of salbutamol

A key part of the community testing was the assessment of reversibility of airway disease. Pellegrino et al recommend use of short acting β_2 agonists such as salbutamol for reversibility testing (Pellegrino et al. 2005). As a prescription only medication (POM), salbutamol is normally administered by prescription. However, the staff undertaking the community visit did not have prescribing rights. I therefore sought alternative solutions to this issue.

Introduced in the UK in 2000 and incorporated in the Human Medicines Regulations, Patient Group Directives (PGD) provide a legal mechanism for qualified health care professionals to administer a POM without the need for a prescription (NHSE 2000). The set-up and use of a PGD is a complicated process. Figure 2-3 outlines the process of developing and implementing a PGD for the study.

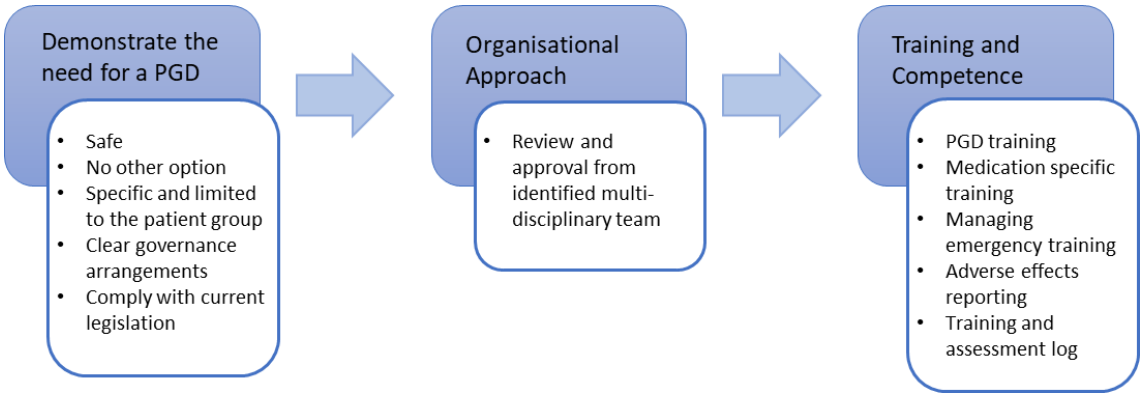


Figure 2-3 Process for developing and implementing a PGD.
Blue boxes outline the steps required to develop a PGD. White boxes outline the actions taken to demonstrate PGD implementation was required and that the necessary governance was in place for use during the study. Adapted from (NICE 2017b).

With no reasonable alternative solution, the use of PGD for the administration of salbutamol for reversibility testing in a community visit was deemed to be the most appropriate. I developed and wrote a PGD which was reviewed and approved by the multi-disciplinary team responsible for safe and effective use of PGDs in the trust. This document can be seen in Appendix 5. I arranged training and assessment of each staff member to ensure that they understood their role in utilising the PGD and the specific documentation related to its use. I also provided training on the specific side effects of β_2 agonists and organised a teaching session with the paediatric asthma nurses to ensure appropriate knowledge and skills were acquired in the safe and effective administration of salbutamol.

2.3 Organising the visit

Three appropriately trained nurses (KH, LY, GW) undertook visits. I trained LY and GW in history taking and clinical examination. I also supervised and supported both nurses in all aspects of organising and undertaking the visits during this project.

On receipt of a questionnaire indicating that the child and family were agreeable to participating in a community visit, the questionnaire was reviewed for completion and to identify any potential exclusion criteria. In cases where children were excluded the family were contacted with an explanation. For those who met inclusion criteria, using the preferred method of contact indicated by the family, a convenient date and time for the visit was arranged. NWIS provided GP details and - as per the lone worker SOP - the child's GP was sent a letter to risk assess the home visit. Two to three days prior to each community visit, the family were contacted to ensure that the child remained well. This also served as a reminder to the family of the date and time of the visit.

Both spirometry and FeNO testing results can be affected by certain medications and foods. Prior to the community visit each child participating in the study was asked to avoid long acting β_2 agonists (LABA), leukotriene receptor agonists and antihistamines for 48 hours and inhaled corticosteroids (ICS) for 24 hours and - where possible - short acting β_2 agonists for 8 hours. All these medications can impact on spirometry results, and the anti-inflammatory effects of leukotriene receptor agonists and corticosteroids can also reduce FeNO measurements.

Participants were also asked to avoid foods containing nitrates/nitrites on the day of the visit as it can artificially increase FeNO measurement results, and to avoid food or drink for one hour prior to the visit commencing due to the potential for causing nausea during spirometry.

2.4 Medical history and clinical examination

As part of a comprehensive assessment, parent reported medical history was collected and a physical examination of each participating child were undertaken prior to lung function testing.

2.4.1 Adapted ISAAC Questionnaire

A modified ISAAC questionnaire - previously used in the RANOP's study - requesting details relating to the child's history of respiratory symptoms and treatment was completed and

returned by the family either prior to, or during, the community visit. These details were checked with the child's parents during the visit to increase the quality and accuracy the information collected. In cases where the questionnaire had been completed greater than 3 months prior to the visit, parents were asked to complete again. This questionnaire can be found in Appendix 6. In cases where data was missing, attempts were made to contact the family to obtain information to ensure data was as complete as possible. In cases where answers to different questions were in conflict (i.e., answered "no" to doctor diagnosis of asthma, but "yes" to inhalers being prescribed and taken), a discussion between team members to identify the most appropriate answer, alongside clear documentation of any changes to answers, was undertaken.

2.4.2 History Taking and Clinical Examination

2.4.2.1 Parent reported medical history

Parent reported medical history pertaining to the child's antenatal and neonatal history, hospital admissions during infancy and childhood and relevant medical treatments were collected during the visit. Prior historical details gained from both the ISAAC questionnaire and NWIS were checked for accuracy with the family. Where available, the child's personal child health record was reviewed for birthweight, gestational age, and relevant neonatal history.

Medical notes were obtained to supplement and check the accuracy of data related to the neonatal period reported by parents and provided by NWIS. Thus, increasing the reliability and validity of information relating to key neonatal events such as gestational age at birth, birthweight, diagnosis of CLD and any documented evidence of co-morbidities such as ROP, IVH and necrotising enterocolitis (NEC). When it was not possible to gain this information, data from NWIS alongside the parental history was used. In cases where there were contradictory reports between NWIS data and parent reported history, or the data was unclear, the team (KH, SK) examined all available information and decided the most likely outcome. Subsequent sensitivity analysis was undertaken by performing data analysis with and without those with history gained from medical notes/discharge summaries to review the reliability and validity of information from children whose notes were inaccessible.

CLD was defined as supplementary oxygen requirement at 28 days of age (<32 weeks' gestation) or 56 days of age/discharge (≥32 weeks' gestation). CLD severity was further defined as mild (CLD_{28}); room air at 36 weeks' gestation PMA (<32 weeks' gestation) or >56

days of age/discharge (≥32 weeks' gestation) and moderate/severe (CLD_{36}); oxygen requirement at 36 weeks' PMA (Jobe and Bancalari 2001).

Birthweight has been used to identify those participants who had evidence of IUGR, defined as birthweight less than the 10th percentile (adjusted for sex and gestation).

Data from the Welsh Index of Multiple Deprivation (WIMD), a ranking system identifying postcodes with the highest level of deprivation (based on eight domains - income, employment, health, education, housing, access to services, community safety, physical environment) has been used to help assess the impact of deprivation on study findings.

2.4.2.2 Physical examination

Each child received a physical examination by a trained staff member. This examination incorporated a comprehensive cardiovascular examination which included a physical examination and non-invasive assessment of peripheral and centra blood pressure with the Vicorder (Smart medical, Gloucester, UK). A thorough respiratory systems evaluation including physical examination and saturation monitoring was also completed alongside a general health check (including temperature) and puberty assessment. Height was measured with a portable stadiometer (Seca 217, Seca deutschland, Hamburg, Germany). Weight and body composition were measured using portable bio-electro impedance scales (TANITA BC-420MA, TANITA Europe B.V., Holland).

2.5 Spirometry

Guidelines and recommendations by the ATS, ERS and British Thoracic Society (BTS) for performing spirometry have standardised equipment specifications, personnel training and testing procedures which ultimately improve the validity and reliability of spirometry testing (Miller et al. 2005). For this reason, they have been used to inform the methods in this study.

Children were recruited in order of descending age, thus enabling the research team to develop of a high level of expertise in eliciting high quality spirometry manoeuvres before testing those at the lower age ranges who presented the greatest risk for poor performance. Children who could not perform baseline spirometry were removed from all analysis.

2.5.1 Equipment

Spirometry was undertaken using a handheld digital bi-directional volume transducer spirometer (Microloop, CareFusion, UK). This device complies with requirements detailed in

guidance produced by the ATS and ERS (Miller et al. 2005). This includes measuring at Body Temperature and Pressure with Saturated water vapour (BTPS). Thus, correcting for the difference between the volume of air in the lungs (37 degrees centigrade) and the volume measured by the spirometer (room temperature) resulting in greater accuracy of test results. In-built technology enabled real-time display of flow vs volume loops and volume vs time. Algorithms were able to immediately analyse both the acceptability and repeatability of tests and could apply appropriate reference ranges to measured raw values. Each testing episode was saved at the time of testing and subsequently uploaded onto a computer system with allied software in the hospital for further quality control and storage.

Calibration with a 3L syringe with a single use mouth filter was performed on each of the four spirometers used for the study as per manufacturers guidelines. Simple flow calibrations were undertaken daily (with measurements within the mid-flow range). More comprehensive weekly flow linearity calibrations were also undertaken, with three consistent tests at low, mid-range and high flows required. Should there be a calibration error outside the recommended variation of ±3%, the calibration would be deemed to have failed. Inspection of the turbine was undertaken, and further calibration attempted. Equipment for which calibration could not be achieved was sent to the manufacturers for review. Daily and weekly calibrations were recorded in a calibration log. A record of equipment sent to the manufacturer was also kept. Additional checks were undertaken with a biological control who tested the spirometers at regular intervals. As per manufacturer's instructions a monthly leak check was undertaken on the calibration syringe.

Single use mouth filters were used for each child to ensure no cross infection occurred. Turbines were removed from their housing and submersed in peri-safe (recommended disinfectant which provides decontamination against a wide range of microbes) for approximately 10 minutes on a weekly basis.

2.5.2 Personnel training

Staff undertaking testing were expert paediatric practitioners. Prior to undertaking the study each team member completed a specialist spirometry course run by the Association for Respiratory Technology and Physiology (ARTP). This two-day course comprised of teaching related to the physiology of respiration, effective spirometry procedures to illicit optimal test results and the interpretation of test results. Following completion of the course, team members practiced teaching spirometry and interpreting output on volunteers. It was felt

that the ARTP course and practice sessions would enable consistent operator performance and high-quality testing. Ongoing feedback was provided to team members about any findings identified during the QC process which may require changes in practice. Training in relation to FeNO testing, reversibility testing and use of all equipment was undertaken by the whole testing team. Nursing staff also received teaching and completed competency assessment in performing a comprehensive respiratory and cardiovascular assessment.

2.5.3 Spirometry testing procedure

The spirometry manoeuvre was explained and subsequently demonstrated to each child who then had the opportunity to practice prior to testing. The procedure was undertaken in a seated position with a nose clip in place. Each child was instructed to breathe out and then place their mouth around a single patient use bacterial filter-ensuring their lips were tightly sealed around the mouthpiece of the filter. They were asked to take in the biggest breath possible and - without hesitation – "blast" the air out as hard and as fast as they could, for as long as possible like they were blowing out candles on a cake (Figure 2-4). To encourage each child to attain their FVC they would be told to "keep going, keep going" until they had completely "run out of puff."

Close observation of each child during the manoeuvre enabled staff to assess the quality of the test performance in real time. Review of the flow vs volume loop and volume vs time graph produced and displayed on the handheld device was undertaken to evaluate individual tests for acceptability. The overlaying of all flow vs volume loops in the spirometer's display also enabled real-time assessment of test repeatability. Table 2-2 outlines the criteria for both acceptability and repeatability as per ATS/ERS guidelines (Miller et al. 2005). Additional assurance of test quality in terms of acceptability and repeatability was provided by in-built software in the spirometer, which also undertook real-time analysis of the tests for both acceptability and reproducibility. This supported local appraisal of spirometry test quality and helped staff provide feedback and further guidance to improve technique.

Due to the unique nature of teaching children, the team adapted their teaching methods to enable each individual child to understand the process. In addition to the available animations in the spirometry device, team members often successfully used party blowers as a teaching tool.

Each child performed the test between three and eight times (Miller et al. 2005) to enable a minimum of three acceptable and reproducible tests results to be obtained. Children who

were unable to perform the procedure were withdrawn from further testing.

Figure 2-4 Child undertaking spirometry.
Microloop handheld digital volume transducer used for spirometry pre- and post-bronchodilator. Written permission from child and parent gained for picture use.

Table 2-2 Criteria for spirometry acceptability and repeatability.
Adapted from Miller (2005). Reproduced with permission.

Within-manoeuvre criteria ***(Individual test criteria for acceptability)***
No evidence of artefacts: Cough during the first second of exhalation Glottis closure that influences the measurement Early termination or cut-off Submaximal effort Leak Obstructed (tongue/teeth) *Good start:* Extrapolated volume >5% of FVC or 0.15 L, whichever is greater *Satisfactory exhalation:* Duration of >3 seconds for children or a plateau in the volume–time curve or If the subject cannot or should not continue to exhale
Between-manoeuvre criteria ***(Criteria for repeatability, once minimum of three acceptable spirograms obtained)***
The two largest values of FVC must be within 0.150 L of each other The two largest values of FEV_1 must be within 0.150 L of each other *or* A total of eight tests have been performed (optional) or The patient/subject cannot or should not continue

2.5.4 Quality control (QC)

In addition to observing the child's performance, reviewing the display of both volume vs flow loops and time vs volume output at the time of testing by both the researcher undertaking the testing and by in-built software in the spirometer, further assessment of the quality of spirometry was performed and review of the results selected by the handheld device was undertaken after the visit had been completed. To ensure the person undertaking the QC process was blinded to the participant details (KH, MC, MW), team members could not QC any tests that they had been directly involved with.

I noted a difference in the selection of individual tests for inclusion by the handheld spirometer and the allied software package in the desktop computer used for uploading data from the spirometer. The difference between the two software packages was related to the time to peak expiratory flow. The computer software excluded tests based on the time to PEF which the handheld unit software did not. This resulted in some individual tests being excluded after the data had been uploaded, altering several test results. As time to PEF is not a defined criteria for acceptability (Miller et al. 2005) it was decided to manually re-evaluate all tests on an individual basis and select the most appropriate results for inclusion. To facilitate this process, I developed a unique QC form which was based on ATS/ERS guidance for standardisation of spirometry (Miller et al. 2005) which would enable consistent and clear selection of tests for use in all data analysis (Appendix 7).

Raw data from the selected tests were extracted and analysed within an Excel document produced by the Global Lung Function Initiative (GLI) to calculate reference values (percent predicted values) (Quanjer 2014b). Differences between the % predicted values generated by the software within the spirometry software and the GLI Excel document were investigated, and any errors found (e.g., incorrect height inputted into spirometer) were corrected across all data records. Cleaned results generated by the GLI Excel document were used in the analysis.

2.5.5 Reference values

Historically, reference values from different populations have been used in the interpretation of spirometry results. Whilst these ranges adjust for differences in measures due to age, height, sex and ethnicity they were largely extrapolated from data from Caucasian males or developed from small numbers of highly select populations. In recognising the limitations of these different reference ranges, the GLI developed a more

robust and comprehensive system which uses collated data from a large pool of datasets from across the world. Despite identifying its own limitations due to lack of data for specific ethnic populations, by combining over forty reference ranges, the GLI has improved the accuracy and consistency of reference ranges for people between 3 and 95 years (Quanjer et al. 2012). To enable appropriate comparison of data in my thesis, GLI generated percent (%) predicted values have been used in the data analysis for FEV_1, FVC and $FEF_{25-75\%}$ whilst the raw values for FEV_1/FVC ratio have been used.

I chose to use a pragmatic cut off point of $\%FEV_1$ value of ≤85% as a proxy for the lower level of normal (LLN) in children 7 to 12 years (Quanjer 2014b) (Table 2-3). This cut off point has been used to identify children with lung function deficits throughout this thesis.

Table 2-3 LLN and comparative % predictive value for FEV_1 by sex and age

Age	Male LLN FEV_1 (L)	Male %predicted FEV_1	Female LLN FEV_1 (L)	Female %predicted FEV_1
7	1.13	79.6	1.06	79.3
8	1.28	80.1	1.20	79.8
9	1.43	80.9	1.36	80.4
10	1.61	81.6	1.53	80.6
11	1.76	81.4	1.72	82.7
12	1.94	80.9	1.96	80.5

LLN and %predicted FEV_1 calculated from GLI reference ranges. Whole ages, Caucasian ethnicity and heights based on 50th percentile from UK-WHO growth charts for males and females were used for calculation purposes.

2.6 Reversibility

Testing for response to bronchodilator was undertaken on all children who successfully completed baseline spirometry and were happy to inhale 400 micrograms (mcg) of salbutamol. The 400mcg of salbutamol was administered to each child under the previously described PGD. Four actuations of 100 mcg of salbutamol were given via a paediatric volumatic spacer device. After each inhaler actuation the child was encouraged to take 10 breaths from the mouthpiece of the spacer device. Spirometry was performed to the same standard as the baseline spirometry 15 minutes after inhalation of the salbutamol. An absolute change in $\%FEV_1$ of >10% was deemed to be evidence of a positive BDR (Quanjer et al. 1993).

2.7 FeNO

FeNO testing is recognised as a useful tool in assisting in the diagnosis and management of asthma in both children and adults (NICE 2014). To help evaluate the role that Th2 driven eosinophilic inflammation plays in the lung function deficits seen in preterm-born children, FeNO testing was undertaken. The NIOX Vero chemiluminescence analyser (Circassia, Oxford, UK), a FeNO testing device which complies with ATS device recommendations for FeNO measurement (Dweik 2011) and is approved by the National Institute for Clinical Excellence (NICE) was used for testing. The children had the procedure explained and then watched a demonstration animation prior to attempting the test. Whilst in a seated position, each child was asked to breath out as much as they could and then - having placed into their mouth an in-built nitric oxide filter - to inhale fully and then breathe out at a steady rate. Animation was used to help each child produce a sustained expiration at a rate of 50 mL/s for 10 seconds (Figure 2-5). Expiration time was reduced to 6 seconds in children who were unable to sustain expiration for 10 seconds. Each child undertook two measurements to ensure consistency in measurements. The highest value of the two tests were used in the analysis.

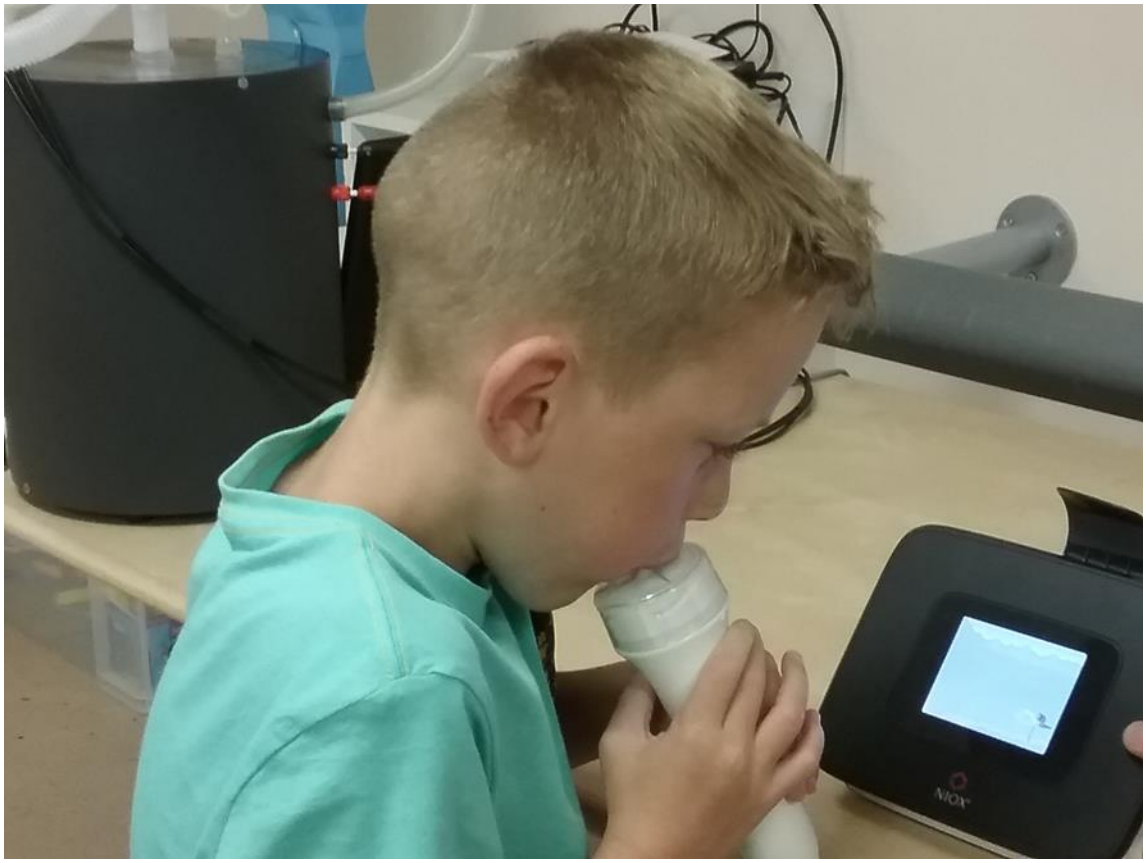


Figure 2-5 Child performing FeNO measurement.
NIOX Vero chemiluminescence analyser with in-built animation used to measure fractional exhaled nitric oxide. Written permission from child and parent gained for picture use.

2.8 Statistical Methods

Several statistical methods were employed during the analysis of data collected.

2.8.1 Sample Size

Sample size calculations for my thesis, developed in consultation with the North Wales Organisation for Randomised Trials in Health Clinical Trials Unit, Bangor (NWORTH CTU), were based on an estimate that the power of the multiple regression model using an alpha of 0.05 and 1 variable for each 100 participants recruited (estimated to 5 – 6 variables) with an R^2 value of 0.2 to be conservative. As this model was being created from scratch with no variables automatically included, there were no controlled independent variables included in the calculation. This model resulted in a power of 100% and so there was sufficient power if all the variables were retained in the model.

The target of 200 of term-born children was based upon the ability to include 3-5 early life factors and a difference of 0.5 standard deviation between the preterm- and term-born groups for FEV_1.

2.8.2 Statistical Analysis

Results for parametric data are presented as means with 95% confidence intervals (95% CI). Non-parametric data are presented as medians and ranges where appropriate. Categorical data are presented as proportions (%). A p-value of <0.05 was deemed to be significant. Results which did not reach the level of statistical significance but were felt to have the potential for clinical significance are also discussed.

Differences in the distribution of categorical demographic data between groups were analysed with Chi-square tests. Additional categorical data developed by the formation of variables based on continuous data e.g. $\%FEV_1$ ≤85% and >85%, birthweight <10th centile, were also analysed with Chi-square tests.

Histograms were used to assess patterns of distribution of continuous data. Parametric data from two groups were analysed with two-tailed independent t-tests. Dependent two-tailed t-tests were used to analyse within group data. One way ANOVA testing with Bonferroni post-hoc analysis was undertaken to analyse parametric data from more than two groups. Non-parametric data were analysed with Mann-Whitney U for two groups, or Kruskall-Wallis for three or more groups.

Exploratory analysis of predictors for childhood lung function deficits was undertaken with univariable and multivariable logistic regression modelling. Continuous data (covariables) are presented with a beta value and p value. Categorical data (factors) are presented with odds ratios (OR) and 95% confidence interval, alongside a p value. Univariables with a $p<0.10$ were deemed significant and used in further multi-variable modelling where a $p<0.05$ was deemed to be significant.

Pearson and Spearman's correlation coefficients for parametric and non-parametric data respectively were used to explore the association between baseline spirometry and both mean change in $\%FEV_1$ post-bronchodilator and FeNO. Assessment of effect size (r) (small 0.10, medium 0.30 and large 0.50) and 95% CI for observed associations are presented. Bootstrapping was used during Spearman's correlation analysis to produce bias corrected (BCa) 95%CI for correlation coefficients. Bootstrapping treats data as non-parametric and by repeatedly sampling from the data (1000 samples in my testing) to gain a sample distribution that overcomes any unequal distribution in the data, enables the production of robust confidence intervals.

Area under the receiver operating characteristic curve (AUROC) analysis was undertaken to assess the diagnostic value of baseline spirometry measures to identify two binary outcomes; those who will have a positive BDR and those who will have a FeNO >35ppb. The receiver operating characteristic curve (ROC) - a probability curve - identifies the sensitivity and specificity of each test to detect a binary outcome ensuring the optimal balance between true positives, false positive, true negatives, and false negatives. The area under the curve (AUC) provided an assessment of the value of each diagnostic test with the following scoring system: 0.5 to 0.59 fail, 0.6 to 0.69 poor, 0.7 to 0.79 fair, 0.8 to 0.89 good, 0.9 to 1.0 very good. Youden's J statistic was used to help identify the cut-off value with the optimal balance between sensitivity and specificity levels. The value of FeNO as a diagnostic tool for predicting those who will have the binary outcome of a positive BDR has also been evaluated using AUROC and Youden's J statistic. This optimal cut-off value is presented for each tested measure alongside its sensitivity, specificity and the diagnostic value score. All statistical analyses were undertaken with SPSS version 23.0 (IBM 2015).

2.9 Ethical Approval

Ethical approval was granted by South-West Bristol Research Ethics Committee (REC Ref 15/SW/0289). The appropriate Health Board research and development departments, alongside Cardiff University research and development department (study sponsor), provided all other relevant permissions. The RHiNO study was funded by the Medical Research Council (MRC) (Reference: MR/M022552/1).

2.10 Informed consent and assent

Mailing specially designed parent information and consent forms (PISC) and child information and assent forms (CISA) along with a letter of invite to participate in the study allowed adequate time for families to consider recruitment prior to participating. Fully informed parent consent and child assent were taken at the beginning of each community visit. In cases where parental consent was granted, but child assent was not obtained the visit did not progress.

3 Results

This results chapter will address the hypotheses and specific aims of my thesis. To enable clear description of findings this chapter is sub-divided into six main sections:

- Recruitment
- Lung function in preterm- and term-born children
- Childhood lung function deficits in preterm-born children and the role of CLD
- Early life factors associated with lung function deficits in the preterm-born population
- Defining respiratory phenotypes in preterm-born children with lung function deficits
- Spirometry and FeNO testing to identify respiratory phenotypes in preterm-born children

3.1 Recruitment

3.1.1 Cohort characteristics

Characteristics of children invited to participate in the study from each separate recruitment cohort (RANOPS, new and term-born controls) are detailed in Table 3-1. The largest number of preterm-born children invited to participate in the study were from the RANOPS cohort.

As expected, both preterm-born cohorts were born at a lower gestational age and birth weight compared with term-born controls. Both preterm-born cohorts also experienced greater deprivation.

Compared with the WIMD rank observed in term-born controls (1053, SD 555), scores in both the new cohort and RANOPs cohort were significantly lower (851, SD 611, $p<0.001$ and 857, SD 576, $p<0.001$). Nearly 50% of the children in both preterm-born groups were from the two most deprived WIMD quintiles. Conversely, 46% of term-born controls were from the two least deprived quintiles. Characteristics of the two preterm-born cohorts were similar except however, the new cohort were older than the RANOPS cohort.

Mean age at the time of invitation of the new cohort (10.5 years, 95%CI 10.4 to 10.6) was significantly higher than both the RANOPS cohort (9.7 years, 95%CI 9.6 to 9.7, $p<0.001$) and term-born controls (10.1 years, 95%CI 10.0 to 10.2, $p<0.001$). The RANOPS cohort were significantly younger than term-born controls ($p<0.001$) with a higher proportion (42.5%) of

children aged 9 years at the time of invitation (Table 3-1, Figure 3-1). The RANOPS group were likely to be younger as this cohort included children who were 2, 3, 4, 6 and 8 years of age in 2013 whereas children from the new cohort were 5, 7 and 9 years in 2013.

Table 3-1 Demographic details of children invited to participate in study

	Preterm-born invitees (n = 3601)		Term-born invitees
	New cohort	RANOPs cohort	
Subjects (n)	1404 (39%)[a]	2197 (61%)[a]	1183
Male	765/1404 (54%)	1208/2197 (55%)	612/1183 (52%)
Age at participation invite	10.5 (10.4 to 10.6) ǂǂ,ƒƒƒ	9.7 (9.6 to 9.7) §§§	10.1 (10.0 to 10.2)
Gestational Age (wks) (range)	32 (20 to 34) ƒƒƒ	32 (23 to 36) §§§	40 (37 to 43)
Birth weight (g) (range)	1779 (1744 to 1815) ƒƒƒ	1802 (1774 to 1830) §§§	3449 (3420 to 3478)
WIMD 2019 (Mean SD)	851 (611) ƒƒƒ	857 (576) §§§	1053 (555)
WIMD 1 (Most Deprived)	337/1101 (31%) ƒƒƒ	604/2197 (27%) §§§	177/1185 (15%)
WIMD 2	234/1101 (21%) ƒ	484/2197 (22%) §§§	211/1185 (18%)
WIMD 3	151/1101 (14%) ƒƒƒ,ǂ	373/2197 (17%)	242/1185 (20%)
WIMD 4	138/1101 (12%) ƒƒƒ,ǂ	338/2197 (15%) §§§	248/1185 (21%)
WIMD 5 (Least Deprived)	241/1101 (22%) ǂǂ	395/2197 (18%) §§§	300/1185 (25%)

[a] Percent of the whole preterm-born cohort.
95% confidence intervals or percentages shown in brackets unless ranges specified.
New cohort vs RANOPS cohort ŧp<0.05, ǂp<0.01, ǂǂp<0.001. New cohort vs TC ƒ p<0.05, ƒ ƒ p<0.01, ƒ ƒ ƒ p<0.001. RANOPS cohort vs TC §p<0.05, §§p<0.01, §§§p<0.001.
Abbreviations: WIMD Welsh Index of Multiple Deprivation.

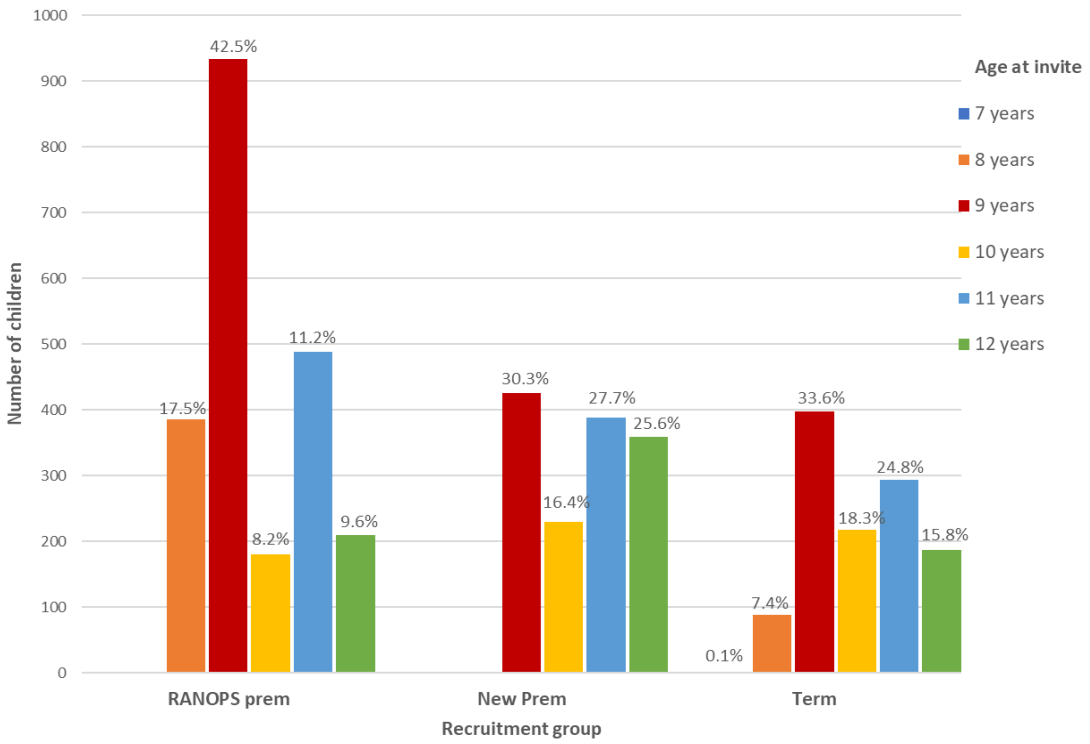


Figure 3-1 Age at invitation by recruitment cohort

Whilst a higher number of preterm-born children were invited to participate, the largest proportion of children receiving an invite to participate in the study were 9 years old in both preterm- (37.8%) and term-born (33.6%) groups. There were also similar proportions of children aged between 9 and 11 years old at the time of invitation in the preterm-born (73.6%) and term-born (76.7%) groups, (Figure 3-2).

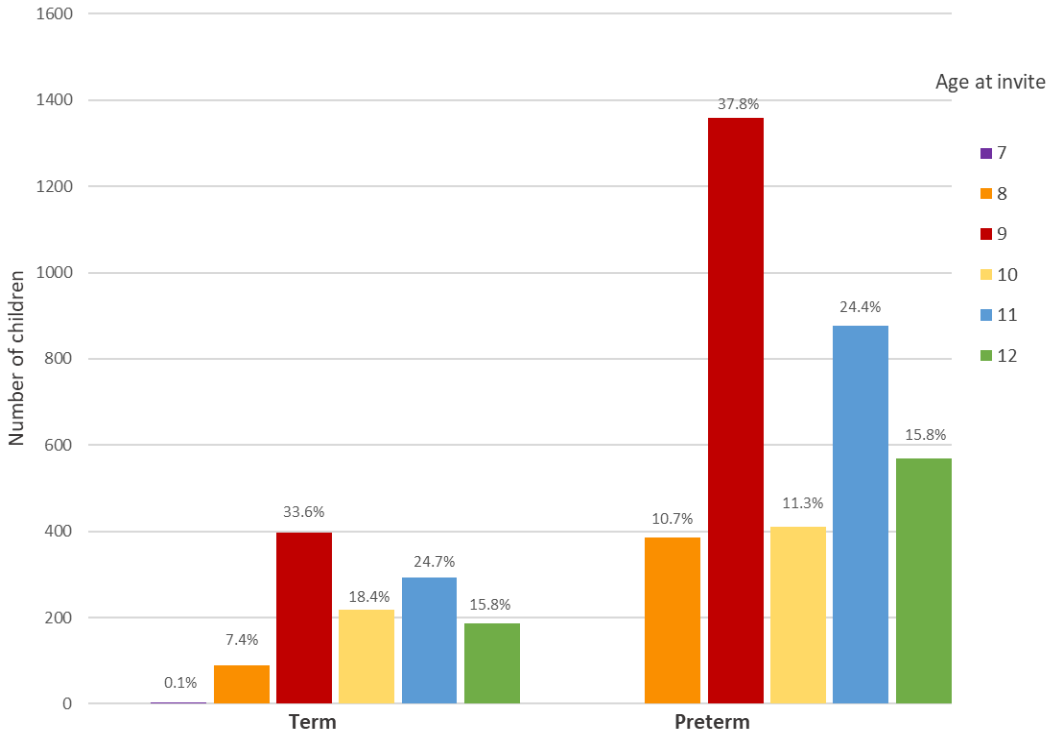


Figure 3-2 Age at invitation in preterm- and term-born children

The CONSORT diagram in Figure 3-3 details the recruitment and flow of participants in the study. In total, 4784 children, (3601 children born at ≤34 weeks' gestation and 1183 children born at >37 weeks' gestation) aged between 7 and 12 years, received an invitation to participate in the study between November 2016 and September 2019.

827 (23%) preterm-born children and 295 (25%) term-born children returned their questionnaire agreeing to participate in a home visit. Despite the nursing team attempting to contact each family who agreed to participate, 197 (24%) preterm-born and 74 (25%) term-born children who had agreed to participate were not contactable. Several children were excluded from testing: 39 (5%) preterm-born (10 incorrect gestation, 28 medical exclusions, 1 currently living outside Wales) and 1 (0.3%) term-born child were excluded due to medical reasons.

A total of 23 children were withdrawn by testing staff during the home visit due to their inability to perform baseline spirometry: 19 (3%) preterm- and 4 (2%) term-born children. The post-visit spirometry QC process also resulted in an additional 28 children (21 (4%) preterm- and 7 (3%) term-born) being removed from further analysis. Overall, 51 (7%) of children agreeing to participate in the study did not perform adequate spirometry. Visits to 21 children could not be arranged due to the study ending before an appointment could be made. In total, 15% of preterm- and 16% of term-born children invited to participate in the study successfully completed testing. Satisfactory data from 739 children (544 preterm- and 195 term-born children) were included in my analysis.

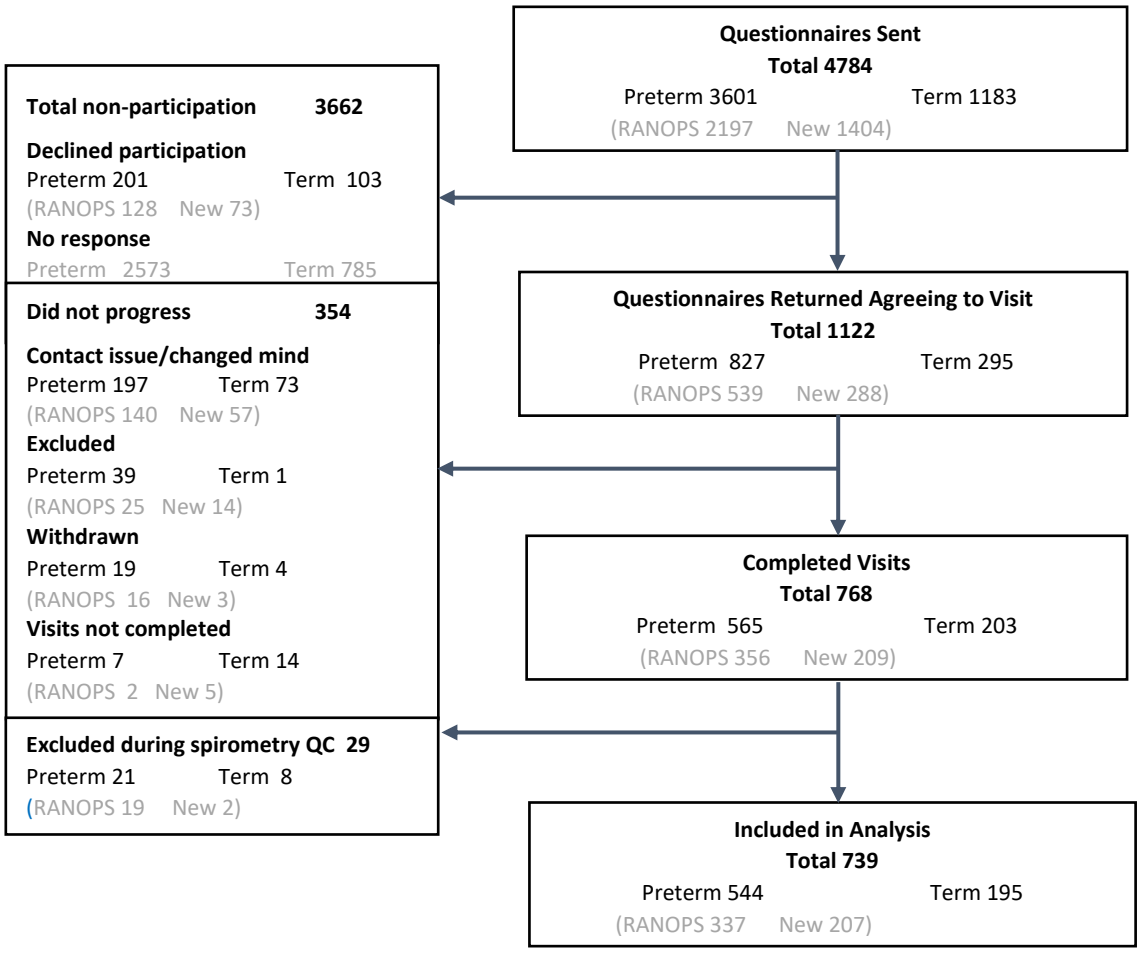


Figure 3-3 CONSORT Diagram.
Details of RANOPS cohort and New cohort in grey text

3.1.2 Responders' v non-responders'

Comparison between demographic details of responders and non-responders in both the preterm-born and term-born groups are detailed in Table 3-2.

Preterm-born responders had a lower birthweight compared with non-responders (1730 gms vs 1820 gms, $p<0.001$). Despite a very small difference in the median gestational age at birth between preterm-born responders and non-responders, it was statistically significant ($p<0.001$). This is likely to be due to the large numbers in the analysis.

Further comparison between preterm-born responders and non-responders showed significant differences in levels of deprivation. Indeed, responders in both groups were less deprived than non-responders. Non-responders had an overall WIMD score suggestive of greater deprivation (1062 vs 813, $p<0.001$). Further analysis also demonstrated a greater proportion of those who did not respond were from the most deprived WIMD score quintile (16% vs 23%, $p<0.001$).

There were no significant differences between the term-born responders and non-responders except a 2% difference in the proportion of male responders (50% vs 52%, $p<0.001$).

Table 3-2 Characteristics of Responders' vs Non responders'

	Preterm-born		Term-born	
	Responders	**Non-Responders**	**Responders**	**Non-Responders**
Subjects (n)	**563**	**3036**	**202**	**983**
Male	293/563 (52%)	1679/3036 (55%)	100/202 (50%) √√√	511/983 (52%)
Gestational Age (wks) (range)	31.6 (23 to 34) √√√	31.4 (20 to 36)	39.6 (37 to 42)	39.5 (37 to 43)
Birth weight (g) (range)	1730 (500 to 3915) √√√	1820 (500 to 4120)	3460 (2300 to 4900)	3460 (1920 to 5030)
WIMD 2019 (Mean SD)	1062 (570) √√√	813 (583)	1192 (522)	1025 (558)
WIMD 1 (Most Deprived)	91/563 (16%) √√√	850/2731 (23%)	16/198 (8%)	161/981 (16%)
WIMD 2	103/563 (18%)	615/2731 (23%)	28/198 (14%)	183/981(19%)
WIMD 3	103/563 (18%)	420/2731 (15%)	41/198 (21%)	202/981 (21%)
WIMD 4	114/563 (20%)	362/2731 (13%)	44/198 (22%)	204/981 (21%)
WIMD 5 (Least Deprived)	152/563 (27%)	484/2731 (18%)	69/198 (35%)	231/981 (24%)
Birth post-smoking ban (2007)	390/547 (69%)	2145/3033 (71%)	157/198 (79%)	769/981 (78%)

95% confidence intervals or percentages shown in brackets unless ranges specified.
Significance √p<0.05, √√p<0.01, √√√p<0.001 comparing responders and non-responders in preterm- and term-born groups.
Abbreviations: WIMD Welsh Index of Multiple Deprivation
Numbers include all responders who consented for a visit, prior to spirometry QC

3.2 Lung function in the preterm- and term-born population

This section describes the characteristics and lung function of preterm-born (PT) school-aged children and describes differences by comparing with term-born controls (TC).

3.2.1 Participant characteristics

In total, 739 children with a mean age of 9.7 years (range 7.0 – 12.0 years) successfully completed baseline spirometry and were include in the analysis. Characteristics of all included participants are detailed in Table 3-3. As expected, compared with the TC group, the PT group were born at lower gestational age (32 weeks' vs 40 weeks', $p<0.001$) and had lower birthweight (1701gms vs 3430gms, $p<0.001$). Despite comparable age and sex divide, subsequent growth has resulted in the PT group being lighter (32.7kg vs 34.8kg, $p<0.05$) and shorter in stature (139.8cms vs 142.0cms, $p<0.01$) than their TC counterparts.

A higher proportion of the PT group were noted to be from the most deprived areas in Wales compared to the TC group (WIMD scores: Mean (SD), 1064 (571) vs 1176 (524), $p<0.05$). There was a higher incidence of both antenatal (12% vs 6%, $p<0.05$) and postnatal maternal smoking (14% vs 4%, $p<0.001$) in the PT group when compared with the TC group. Whilst a high proportion of both PT and TC children were born after the introduction of the Welsh smoking ban in 2007, this applied to a significantly higher proportion of the TC group compared with the PT group (79% vs 69%, $p<0.001$).

There was also a higher incidence of IUGR (14% vs 5%, $p<0.01$) and delivery by caesarean section (57% vs 25%, $p<0.001$) in the PT group. Unsurprisingly, the PT group had greater incidence of neonatal illness. In total, 108 (20%) PT children had a prior diagnosis of CLD, with 14 (3%) requiring postnatal corticosteroids and 25 (5%) requiring home oxygen.

A higher incidence of historical and persisting respiratory symptoms was reported in the PT group compared with the TC group. The PT group reported higher incidence of bronchiolitis (18% vs 5%, $p<0.001$), viral-induced wheeze (10% vs 4%, $p<0.01$) and wheeze-ever (54% vs 27%, $p<0.001$). Despite no difference between groups in family history of atopy, the PT group reported greater current respiratory symptoms and treatment compared to the TC group. Whilst 28% of the PT group reported wheeze in the last 12 months, only 17% have received inhaled treatment with a similar proportion (16%) having a diagnosis of asthma.

Table 3-3 Characteristics of preterm- and term-born study participants

	Whole population n=739, mean age 9.7 years	
	≤34/40 Preterm-born (PT)	**≥37/40 Term-born controls (TC)**
Subjects (n)	544 (74%)	195 (26%)
Current status		
Male	279/544 (51%)	100/195 (51%)
Current Age (y)	9.7 (9.5 to 9.8)	9.7 (9.5 to 9.8)
Current Height (cm)	139.8 (138.9 to 141.7) √√	142.0 (140.7 to 143.3)
Current Weight (kg) (range)	32.7 (17.7 to 88.9) √	34.8 (21.4 to 78.2)
Current BMI (range)	17.0 (12.2 to 32.5)	17.1 (13.2 to 30.9)
WIMD 2019 (Mean SD)	1064 (571)√	1176 (524)
Born post-smoking ban (2007)	79%√√	69%
Neonatal History		
Gestational Age (wks) (range)	32 (23 to 34) √√√	40 (37 to 42)
Birth weight (g) (range)	1701 (450 - 3912) √√√	3430 (2155 to 4916)
Birthweight adjusted z-score	0.167 (0.053 to 0.281)	0.062 (-0.028 to 0.199)
IUGR	76/544 (14%) √√	9/195 (5%)
PROM	201/530 (38%) √√√	6/185 (3%)
C/S delivery	306/542 (57%) √√√	49/195 (25%)
Antenatal corticosteroids	451/511 (88%) √√√	4/195 (2%)
Postnatal corticosteroids	14/514 (3%) √	0/194 (0%)
CLD	108/544 (20%) √√√	0/195 (0%)
Home oxygen	25/535 (5%) √√	0/194 (0%)
ROP	32/544 (6%) √√	0/195 (0%)
Chest drain	19/530 (4%) √√	0/193 (0%)
IVH	56/544 (10%) √√√	0/195 (0%)
NEC	31/527 (6%) √√	0/194 (0%)
PDA	35/526 (7%) √√√	0/195 (0%)
Combined Illness	94/530 (18%) √√√	0/194 (0%)
Family History		
Maternal antenatal smoking	62/532 (12%) √	11/194 (6%)
Maternal postnatal smoking	74/539 (14%) √√√	8/195 (4%)
F/H Asthma	296/539 (55%)	91/195 (47%)
F/H Hay fever	289/534 (54%)	115/194 (59%)
F/H Eczema	242/531 (46%)	90/195 (46%)
F/H Allergies	208/531 (39%)	81/193 (42%)
Respiratory symptoms		
Bronchiolitis	97/542 (18%) √√√	10/194 (5%)
Viral-induced wheeze	52/541 (10%) √√	7/195 (4%)
Pneumonia	34/542 (6%)	6/194 (3%)
Wheeze-ever	281/523 (54%) √√√	51/191 (27%)
Wheeze last 12 months	152/544 (28%) √√√	25/195 (13%)
Inhalers last 12 months	93/544 (17%) √√√	12/195 (6%)
Diagnosed Asthma	88/543 (16%) √√√	10/193 (5%)

95% confidence intervals or percentages shown in brackets unless ranges specified. Adjusted birthweight z-score - adjustments for gestation and sex. **Significance √p<0.05, √√p<0.01, √√√p<0.001**. 2019 WIMD used to calculate proportion of those in each group in the most deprived areas.

Abbreviations: PROM = prolonged rupture of membranes, C/S = caesarean section delivery, CLD = Chronic lung disease, ROP = retinopathy of prematurity, NEC = necrotising enterocolitis requiring treatment, PDA = patent ductus arteriosus requiring medical or surgical treatment, Combined Illness = IVH or ROP or NEC in the neonatal period, Chest drain = insertion to treat pneumothorax, FH = family history, WIMD Welsh Index of Multiple Deprivation.

3.2.2 Lung function in the preterm-born population

Of the 739 participants who successfully completed baseline spirometry, 544 were preterm-born and 195 term-born. Forty children (5%) did not complete post-bronchodilator spirometry: 28 (5%) in the PT group and 12 (6%) in the TC group. Several children were unable to perform FeNO testing: 70 (13%) in the PT group and 12 (6%) in the TC group.

Results detailed in Table 3-4 demonstrate that compared to the TC group, all baseline spirometry measures were lower in the PT group. The baseline $\%FEV_1$ was significantly lower (mean 91.2% vs 95.7%, $p<0.001$) but remained within acceptable parameters. However, 26% of the PT group had an $\%FEV_1 \leq 85\%$ compared with 16% of the TC group ($p<0.01$) suggesting a higher level of children in the preterm-born group had clinically significant deficits in $\%FEV_1$.

3.2.3 BDR and FeNO

Despite the PT group having the greater increase in $\%FEV_1$ post-bronchodilator (5.3% vs 3.6%, $p<0.01$), it remained lower than the TC group (mean 96.4% vs 99.0%, $p<0.05$) after bronchodilator. Similarly, whilst there were significant increases in FEV_1/FVC ratio (0.038 vs 0.029, $p<0.05$) and $\%FEF_{25-75\%}$ (13.9 vs 11.0, $p<0.01$) in the PT group, they remained lower than the TC group.

As expected, a higher proportion of PT children had a positive BDR, defined as an absolute increase in $\%FEV_1$ >10% (13% vs 6%, $p<0.01$). Despite this, there were no differences in the proportions of children in each group with clinically relevant FeNO >35ppb (13% vs 11%, p=NS).

Table 3-4 Lung function measures in preterm- and term-born children

	≤34/40 Preterm-born (PT)	**≥37/40 Term-born control (TC)**
Baseline spirometry	**n = 544**	**n = 195**
% predicted FEV_1	91.2 (90.1 to 92.2) √√√	95.7 (94.2 to 97.0)
% predicted FVC	94.3 (93.3 to 95.3) √	96.2 (94.8 to 97.7)
FEV_1/FVC ratio	0.85 (0.84 to 0.85) √√√	0.87 (0.86 to 0.88)
% predicted $FEF_{25-75\%}$	77.0 (75.2 to 78.7) √√√	86.4 (83.6 to 89.1)
Post-BD spirometry	**n = 516**	**n = 183**
% predicted FEV_1	96.4 (95.4 to 97.5) √	99.0 (97.5 to 100.5)
% predicted FVC	95.7 (94.7 to 96.7)	96.7 (95.2 to 98.3)
FEV_1/FVC ratio	0.88 (0.88 to 0.89) √	0.89 (0.89 to 0.90)
% predicted $FEF_{25-75\%}$	90.5 (88.7 to 92.3) √√	96.2 (93.4 to 99.1)
Mean Change in spirometry ##	**n = 516**	**n = 183**
% predicted FEV_1	5.3 (4.8 to 5.8) √√	3.6 (2.9 to 4.3)
% predicted FVC	1.2 (0.7 to 1.7)	0.5 (-0.1 to 1.1)
FEV_1/FVC ratio	0.038 (0.034 to 0.042) √	0.029 (0.023 to 0.035)
% predicted $FEF_{25-75\%}$	13.9 (12.8 to 14.9) √√	11.0 (8.9 to 13.0)
Baseline %FEV_1 ≤85% predicted value	141 (26%) √√	31 (16%)
Positive BDR	66 (13%) √√	10 (6%)
Fractional exhaled nitric oxide (FeNO)	**n = 474**	**n = 183**
FeNO >35ppb	61 (13%)	20 (11%)

95% confidence intervals or percentages shown in brackets. **Significance √p<0.05, √√p<0.01, √√√p<0.001.**
Mean change in spirometry readings based on all children with pre- and post-BD spirometry.

3.2.4 Summary of results

These data so far show that, in addition to lower gestation and birthweight, the preterm-born population had higher incidence of IUGR, delivery by caesarean section, respiratory illness and poorer general health in the neonatal period. Compared with term-born controls, a greater proportion of the preterm-born group were exposed to maternal antenatal and postnatal smoking. This may be due to a higher proportion of the preterm-born group being born prior to the introduction of the Welsh smoking ban or associated with greater levels of deprivation.

Preterm-born children are at greater risk for lung function deficits in childhood compared to term-born counterparts. They had lower spirometry measures, reported higher rates of respiratory symptoms, and demonstrated greater responses to a single dose of inhaled bronchodilator.

Whilst comparison between the preterm- and term-born population showed differences in lung function, this simplistic approach does not clearly identify those at increased risk for significant lung function deficits in childhood and those who may benefit from inhaled therapies. There is a need to accurately identify those at highest risk for clinically significant lung function impairment, evaluate their response to bronchodilator and identify any role that FeNO may have. One disease process that may be associated with the increased risk for lung function deficits in childhood is CLD, which I explored next.

3.3 Childhood lung function deficits in the preterm-born population and the role of CLD

CLD has historically been used as a predictor for later life lung function deficits. The pathology of CLD has evolved from one of abnormal fibrotic lungs with areas of collapse and hyperinflation to one of larger simplified alveoli with altered architecture. Whilst diagnostic criteria have been adapted to reflect this fundamental change in the pathology of the disease, it may be that progression of clinical management may have resulted in further evolution of disease pathology resulting in existing adaptations to diagnostic criteria already being outdated. The potential evolution of CLD pathology and inconsistent application of diagnostic criteria have led to others questioning the value of CLD in predicting lung function deficits in graduates of modern neonatal care (Corwin et al. 2018). Despite these questions, I hypothesised that preterm-born children with a history of CLD in infancy have a greater incidence of lung function deficits compared to term- and preterm-born children with no history of CLD.

To understand the impact of CLD on childhood lung function in this cohort of children born in the post-surfactant era, I analysed the characteristics and lung function of the preterm-born group by diagnosis of CLD, comparing those with CLD (CLD), defined as supplemental oxygen requirement at 28 of days age, those without CLD (No-CLD) and term-born controls (TC).

Lung function deficits have also previously been associated with the severity of CLD (Shennan et al. 1988). With an adequate number of children with CLD in the cohort, I took the opportunity to undertake sub-group analysis of those with mild CLD based on oxygen requirement limited to 28 days of age (CLD_{28}), or moderate/severe CLD (CLD_{36}) based on an oxygen requirement at 36 weeks' PMA for those born <32 weeks' gestation or having an oxygen requirement at 56 days of age or at discharge for those born ≥32 weeks' gestation.

This section examines the role of CLD on lung function compared with preterm-born children without CLD and term-born controls. It evaluates the impact that disease severity has on lung function and describes the response to bronchodilator in those with CLD compared with those without CLD and term-born controls. The role of Th2 driven eosinophilic inflammation is also evaluated.

3.3.1 Characteristics of participants by CLD status

Table 3-5 describes the participant demographics by CLD status. In total, 739 children (544 preterm- and 195 term-born) successfully completed baseline spirometry. Of the 544 preterm-born participants, 108 (20%) had CLD; 40 (37%) had CLD_{28} and 68 (63%) CLD_{36}.

The CLD group were shorter than those in the No-CLD group (138.2cms vs 140.2cms, $p<0.05$). The CLD group were also lighter than the TC group (30.8kg vs 34.8kgs, $p<0.01$) and shorter than the TC group, although this was not statistically significant.

Predictably, both preterm-born groups were born at earlier gestations and had lower birth weights compared to the TC group. Compared with the TC group, median gestational age of 40 weeks', both preterm-born groups were born earlier - the No-CLD group at 32 weeks' gestation ($p<0.001$) and the CLD group at 27 weeks' gestation ($p<0.001$). The CLD group were born at significantly lower gestation compared with the No-CLD group ($p<0.001$).

Both preterm-born groups also had lower birthweights compared with the mean birthweight of 3430 grams observed in the TC group - No-CLD group 1843 grams ($p<0.001$) and CLD group 946 grams ($p<0.001$). Again, there was a significant difference between those with and without CLD, with the CLD group having the lowest birthweight ($p<0.001$).

Both preterm-born groups had lower WIMD scores compared to the TC group (Mean (SD), No-CLD 1048 (571), CLD 1127 (567), TC 1176 (524)). However, the only significant difference was between the No-CLD and TC group ($p<0.05$). A greater proportion of the TC group were born after 2007 compared with No-CLD (79% vs 70%, $p<0.05$) and CLD (79% vs 64%, $p<0.01$) groups.

The rates of prolonged rupture of membranes (PROM), administration of maternal antenatal corticosteroids and delivery by caesarean section were higher in both CLD and No-CLD groups compared to the TC group. Both CLD and No-CLD groups had higher incidence of IUGR compared to the TC group (18% vs 5%, $p<0.001$ and 13% vs 5%, $p<0.01$ respectively).

As expected, compared with the TC group, both preterm-born groups had increased rates of illness during the neonatal period. Compared to the No-CLD group, the CLD group had received more postnatal corticosteroids (15% vs 0%, $p<0.001$) and more were discharged home on oxygen (23% vs 0%, $p<0.001$). The CLD group had greater neonatal morbidity when compared to the No-CLD group: ROP (22% vs 2%, $p<0.001$), IVH (30% vs 6%, $p<0.001$), NEC (20% vs 2%, $p<0.001$) and chest drain insertions (7% vs 3%, $p<0.01$). Overall, 52% of the CLD

group compared with 9% of the No-CLD group had combined illness (PDA, NEC, or ROP) ($p<0.001$).

Having earlier described similar proportions of those with a family history of atopy between the whole PT and TC groups, analysis based on CLD status revealed that the No-CLD group had a higher proportion of family history of asthma when compared with the TC group (56% vs 47%, $p<0.05$), but not the CLD group. All other measures of family history of atopy were comparable between all groups.

The increased proportion of preterm-born children exposed to maternal antenatal and postnatal smoking compared with term-born controls has previously been described (Moore et al. 2016). Whilst the rates of maternal smokers were similar between both the CLD and No-CLD groups, both preterm-born groups had higher maternal antenatal and postnatal smoking compared to the TC group.

Table 3-5 Characteristics of participants based on CLD status

	≤34/40 with CLD (CLD)	≤34/40 with CLD at 28 days (CLD_{28})	≤34/40 with CLD at 36 weeks' (CLD_{36})	≤34/40 without CLD (No-CLD)	≥37/40 Term-born controls (TC)
Subjects (n)	108 (20%) [a]	40 (37%) [b]	68 (63%) [b]	436 (80%) [a]	195 (26%) [c]
Current status					
Male	57/108 (53%)	25/40 (63%)	32/68 (47%)	222/436 (51%)	100/195 (51%)
Current Age (y)	9.7 (9.4 to 10.0)	9.5 (9.0 to 9.9)	9.9 (9.5 to 10.2)	9.6 (9.5 to 9.8)	9.7 (9.5 to 9.8)
Current Height (cm)	138.2 (136.1 to 140.3) ¥	138.0 (134.6 to 141.4)	138.3 (135.5 to 141.1)	140.2 (139.2 to 141.1)	142.0 (140.7 to 143.3)
Current Weight (kg) (range)	30.8 (17.7 to 72.3) **	29.8 (17.7 to 72.3)	32.4 (19.4 to 62.4)	33.0 (18.2 to 88.9)	34.8 (21.4 to 78.2)
Current BMI (range)	16.7 (12.8 to 30.5)	16.2 (13.6 to 30.5)	17.2 (12.8 to 26.4)	17.0 (12.2 to 32.5)	17.1 (13.2 to 30.9)
WIMD 2019 (Mean SD)	1127 (567)	1210 (561)	1077 (568)	1048 (571) ¢	1176 (524)
Born post-smoking (2007)	69/108 (64%) **	26/40 (65%)	43/68 (63%) †	304/436 (70%)¢	153/195 (79%)
Neonatal history					
Gestational Age (wks) (range)	27 (23 to 32) ¥¥¥, ***	28 (24 to 31) ΨΨΨ, ΔΔΔ	27 (23 to 32) ≠≠≠, †††	32 (26 to 34) ¢¢¢	40 (37 to 42)
Birth weight (g) (range)	946 (450 to 2300) ¥¥¥, ***	1117 (652 to 1700) ΨΨΨ, ΔΔΔ	905 (450 to 2300) ≠≠≠, †††	1843 (500 to 3912) ¢¢¢	3430 (2155 to 4916)
Birthweight Adjusted z-score	-0.054 (-0.290 to 0.187)	0.094 (-0.243 to 0.432)	-0.142 (-0.464 to 0.180)	0.220 (0.092 to 0.352)	0.062 (-0.028 to 0.199)
IUGR	19/108 (18%) ***	3/40 (8%)	16/68 (24%) ϟ, ≠, †††	57/436 (13%) ¢¢	9/195 (5%)
PROM	34/108 (32%) ***	15/40 (38%) ΔΔΔ	19/68 (28%) †††	167/422 (40%) ¢¢¢	6/185 (3%)
C/S delivery	53/108 (49%) ***	16/40 (40%) Ψ	37/68 (54%) †††	253/434 (58%) ¢¢¢	49/195 (25%)
Antenatal steroids	94/103 (91%) ***	34/38 (90%) ΔΔΔ	60/65 (92%) †††	357/408 (88%) ¢¢¢	4/195 (2%)
Postnatal corticosteroids	14/95 (15%), ***	2/38 (5%) ΨΨ, Δ	12/57 (21%) ϟ, ≠≠≠, †††	0/419 (0%)	0/194 (0%)
Home oxygen	25/107 (23%) ¥¥¥, ***	0/39 (0%)	25/68 (37%) ϟϟϟ, ≠≠≠, †††	0/428 (0%)	0/194 (0%)
ROP	24/108 (22%)¥¥¥, ***	7/40 (18%) ΨΨΨ, ΔΔΔ	17/68 (25%) ≠≠≠, †††	8/436 (2%)	0/195 (0%)
Chest drain	7/106 (7%) **	3/39 (8%) ΔΔ	4/67 (6%) ††	12/424 (3%) ¢	0/193 (0%)
IVH	32/108 (30%)¥¥¥, ***	13/40 (33%) ΨΨΨ, ΔΔΔ	19/68 (28%) ≠≠≠, †††	24/436 (6%) ¢¢	0/195 (0%)
NEC	21/105 (20%)¥¥¥, ***	4/38 (11%) Ψ, ΔΔ	17/67 (25%) ≠≠≠, †††	10/422 (2%) ¢	0/194 (0%)
PDA	32/105 (31%)¥¥¥, ***	8/37 (22%) ΨΨΨ, ΔΔΔ	24/68 (35%) ≠≠≠, †††	3/421 (1%)	0/195 (0%)
Combined Illness	56/107 (52%) ¥¥¥, ***	18/39 (46%) ΨΨΨ, ΔΔΔ	38/68 (56%) ≠≠≠, †††	38/423 (9%) ¢¢¢	0/194 (0%)

Family History					
Maternal antenatal Smoking	15/108 (14%) *	3/40 (8%)	12/68 (18%) ††	47/424 (11%) ¢	11/194 (6%)
Maternal postnatal smoking	16/108 (15%) **	5/40 (13%)	11/68 (16%) ††	58/431 (14%) ¢¢¢	8/195 (4%)
F/H Asthma	53/108 (49%)	18/40 (45%)	35/68 (52%)	243/431 (56%) ¢	91/195 (47%)
F/H Hay fever	51/106 (48%)	17/38 (45%)	34/68 (50%)	238/428 (56%)	115/194 (59%)
F/H Eczema	43/106 (41%)	14/38 (37%)	29/68 (43%)	199/425 (47%)	90/195 (46%)
F/H Allergies	35/106 (33%)	13/38 (34%)	22/68 (32%)	173/425 (41%)	81/193 (42%)

95% confidence intervals or percentages shown in brackets unless ranges specified. Adjusted birthweight z-score - adjustments for gestation and sex. [a] =% preterm-born population, [b] =%preterm-born population with CLD, [c] = % of total population.

CLD vs No-CLD ¥ p<0.05, ¥¥ p<0.01, ¥¥¥p<0.001 **CLD vs TC *p<0.05, ** p<0.01, *** p<0.001**
No-CLD vs TC ¢p<0.05, ¢¢p<0.01, ¢¢¢p<0.001 **CLD_{36} vs CLD_{28} ϟp<0.05, ϟϟp<0.01, ϟϟϟp<0.001,**
CLD_{36} vs No-CLD ≠p<0.05, ≠≠p<0.01, ≠≠≠p<0.001 **CLD_{36} vs TC †p<0.05, ††p<0.01, †††p<0.001**
CLD_{28} vs No-CLD Ψp<0.05, ΨΨp<0.01, ΨΨΨp<0.001 **CLD_{28} vs TC Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001**

Abbreviations: PROM = prolonged rupture of membranes, C/S = caesarean section delivery, CLD =Chronic lung disease, ROP = retinopathy of prematurity, NEC = necrotising enterocolitis requiring treatment, PDA = patent ductus arteriosus requiring medical or surgical treatment, Combined Illness = IVH or ROP or NEC in the neonatal period, Chest drain = insertion to treat pneumothorax, FH = family history.

Having previously described higher reporting of respiratory symptoms in preterm-born children compared with term-born controls, analysis of preterm-born children by CLD status showed a gradient in rates of bronchiolitis with the highest rates reported in the CLD group, intermediate rates in the No-CLD group and the lowest rates in the TC group (29%, 15% and 5% respectively), (Table 3-6). Similarly, both the CLD and No-CLD groups reported greater prevalence of wheeze-ever compared to the TC group (68%, 50% and 27% respectively). The CLD group had the highest incidence of bronchiolitis compared to both the No-CLD ($p<0.01$) and TC groups ($p<0.001$) and the highest prevalence of wheeze-ever compared to the No-CLD ($p<0.01$) and TC ($p<0.001$) groups.

Both preterm-born groups reported higher wheeze in the last 12 months compared with term-born controls. Unlike wheeze-ever, the incidence of wheeze in the last 12 months dropped to the same level of 28% in both the CLD and No-CLD groups. The proportions of children with a diagnosis of asthma and those who have received inhalers in the last 12 months were also comparable between the two preterm-born groups.

Table 3-6 Postnatal respiratory health by CLD status

	≤34/40 with CLD (CLD)	≤34/40 with CLD at 28 days (CLD_{28})	≤34/40 with CLD at 36 weeks' (CLD_{36})	≤34/40 without CLD (No-CLD)	≥37/40 Term-born controls (TC)
Subjects (n)	108 (20%) [a]	40 (37%) [b]	68 (63%) [b]	436 (80%) [a]	195 (26%) [c]
Bronchiolitis	31/108 (29%) ¥¥¥, ***	11/40 (28%) Ψ, ΔΔΔ	20/68 (29%) ≠≠, †††	66/434 (15%) ¢¢	10/194 (5%)
Viral-induced wheeze	9/107 (8%)	4/40 (10%)	5/67 (8%)	43/436 (10%) ¢¢	7/195 (4%)
Pneumonia	12/108 (12%) ¥¥, **	6/40 (15%) Ψ, ΔΔ	7/68 (10%) †	21/434 (5%)	6/194 (3%)
Wheeze-ever	72/106 (68%) ¥¥, ***	23/39 (59%) ΔΔΔ	49/67 (73%) ≠≠, †††	209/417 (50%) ¢¢¢	51/191 (27%)
Wheeze last 12 months	30/108 (28%) **	8/40 (20%)	22/68 (32%) ††	122/436 (28%) ¢¢¢	25/195 (13%)
Inhalers last 12 months	15/108 (14%) *	7/40 (18%) Δ	8/68 (12%)	78/436 (18%) ¢¢¢	12/195 (6%)
Diagnosed Asthma	18/108 (17%) **	8/40 (20%) ΔΔ	10/68 (15%) †	70/435 (16%) ¢¢¢	10/193 (5%)

95% confidence intervals or percentages shown in brackets unless ranges specified. Adjusted birthweight z-score - adjustments for gestation and sex. [a] =% preterm-born population, [b] =%preterm-born population with CLD, [c] = % of total population.

CLD vs No-CLD ¥ p<0.05, ¥¥ p<0.01, ¥¥¥p<0.001 **CLD vs TC *p<0.05, ** p<0.01, *** p<0.001**
No-CLD vs TC ¢p<0.05, ¢¢p<0.01, ¢¢¢p<0.001 **CLD_{36} vs CLD_{28} ϟp<0.05, ϟϟp<0.01, ϟϟϟp<0.001,**
CLD_{36} vs No-CLD ≠p<0.05, ≠≠p<0.01, ≠≠≠p<0.001 **CLD_{36} vs TC †p<0.05, ††p<0.01, †††p<0.001**
CLD_{28} vs No-CLD Ψp<0.05, ΨΨp<0.01, ΨΨΨp<0.001 **CLD_{28} vs TC Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001**

3.3.2 Lung function by CLD status

Successful baseline spirometry was completed by 108 children with CLD, 436 children with No-CLD and 195 term-born controls. 3 (3%) children from the CLD group, 25 (6%) children from the No-CLD group and 12 (6%) children from the TC group did not perform post-bronchodilator spirometry. Additionally, 24 (22%) children from the CLD group, 46 (11%) children from the No-CLD and 12 (6%) children from the TC group could not perform FeNO.

All baseline spirometry measures were lower in the CLD group compared to both the No-CLD and TC groups (Table 3-7). The CLD group had the lowest baseline $\%FEV_1$ compared to both the TC (86.2% vs 95.7%, $p<0.001$) and No-CLD groups (86.2% vs 92.4%, $p<0.001$). However, the No-CLD group also had lower $\%FEV_1$ when compared with the TC group (92.4% vs 95.7%, $p<0.01$). Additional measures of lung function impairment - FEV_1/FVC ratio and $\%FEF_{25-75\%}$ were also reduced in both the CLD and No-CLD groups compared with the TC group.

3.3.3 BDR and FeNO

Those with CLD had the largest increases in all spirometry measures following inhalation of bronchodilator except for $\%FEF_{25-75\%}$, which was noted to have increased by a similar percent in all three groups. The post-bronchodilator increases in the CLD group resulted in a %FVC which was comparable to the No-CLD and TC groups. However, $\%FEV_1$, FEV_1/FVC and $\%FEF_{25-75\%}$ measures in the CLD group remained lower than both comparison groups. The No-CLD group had $\%FEV_1$, FEV_1/FVC and $\%FEF_{25-75\%}$ measures that were comparable with the TC group after bronchodilator(Table 3-7, Figure 3-4).

The CLD group had the lowest baseline spirometry measures and the largest proportion of children with a positive BDR (19%). However, those in the No-CLD group also had reduced baseline spirometry and a higher proportion of children with a positive BDR (11%) compared with the TC group (6%). Despite the No-CLD group having a significantly higher proportion of family history of asthma compared to the TC group (56% vs 47%, $p<0.05$), the proportions of children with a high level of FeNO was similar across all three groups.

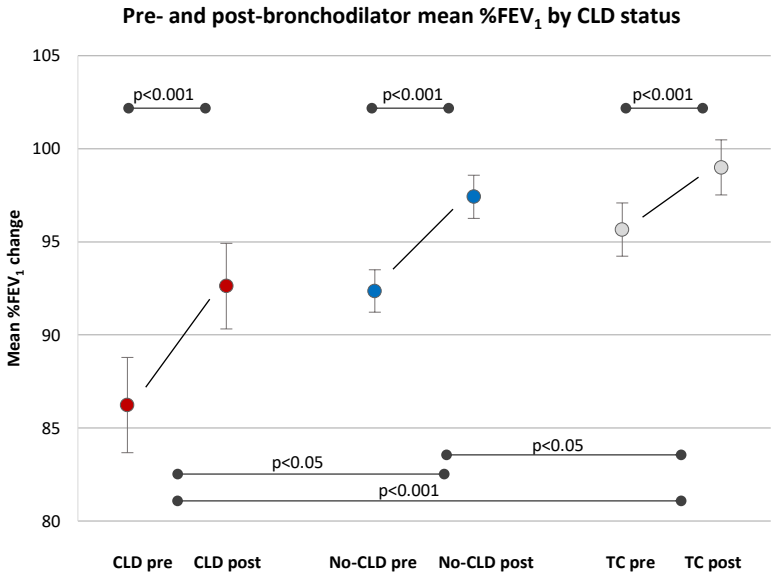
Table 3-7 Lung function by CLD status

	≤34/40 with CLD (CLD)	≤34/40 with CLD at 28 days (CLD_{28})	≤34/40 with CLD at 36 days (CLD_{36})	≤34/40 without CLD (No-CLD)	≥37/40 Term-born control (TC)
Baseline spirometry	**n = 108**	**n = 40**	**n = 68**	**n = 436**	**n = 195**
% predicted FEV_1	86.2 (83.7 to 88.8) ¥¥¥, ***	88.7 (84.9 to 92.5) ΔΔ	84.8 (81.4 to 88.2) ≠≠≠, †††	92.4 (91.2 to 93.5) ¢¢	95.7 (94.2 to 97.0)
% predicted FVC	92.0 (89.8 to 94.1) ¥, **	92.3 (88.9 to 95.7)	91.8 (89.0 to 94.5) †	94.9 (93.8 to 95.9)	96.2 (94.8 to 97.7)
FEV_1/FVC ratio	0.82 (0.80 to 0.84) ¥¥¥, ***	0.84 (0.82 to 0.86)	0.81 (0.79 to 0.83) ≠≠≠, †††	0.85 (0.85 to 0.86) ¢	0.87 (0.86 to 0.88)
% predicted $FEF_{25-75\%}$	68.7 (64.6 to 72.8) ¥¥¥, ***	73.6 (67.3 to 79.9) ΔΔ	65.8 (60.5 to 71.1) ≠≠≠, †††	79.0 (77.1 to 80.9) ¢¢¢	86.4 (83.6 to 89.1)
Post-BD spirometry	**n = 105**	**n = 38**	**n = 67**	**n = 411**	**n = 183**
% predicted FEV_1	92.6 (90.3 to 94.9) ¥¥¥, ***	94.0 (90.5 to 97.5)	91.8 (88.8 to 94.9) ≠≠, †††	97.4 (96.3 to 98.6)	99.0 (97.5 to 100.5)
% predicted FVC	94.2 (92.1 to 96.3)	93.5 (90.1 to 96.9)	94.6 (91.9 to 97.3)	96.1 (95.0 to 97.2)	96.7 (95.2 to 98.3)
FEV_1/FVC ratio (95% CI)	0.86 (0.85 to 0.88) ¥¥, ***	0.88 (0.86 to 0.89)	0.85 (0.83 to 0.87) ≠≠≠, †††	0.89 (0.88 to 0.89)	0.89 (0.89 to 0.90)
% predicted $FEF_{25-75\%}$	82.4 (78.2 to 86.7) ¥¥¥, ***	87.2 (80.9 to 93.4)	79.8 (74.2 to 85.3) ≠≠≠, †††	92.5 (90.6 to 94.5)	96.2 (93.4 to 99.1)
Mean Change in spirometry ##	**n = 105**	**n = 38**	**n = 67**	**n = 411**	**n = 183**
% predicted FEV_1	6.6 (5.2 to 7.9) ¥, ***	6.0 (4.2 to 7.7)	6.9 (5.1 to 8.8) †††	4.9 (4.4 to 5.5) ¢	3.6 (2.9 to 4.3)
% predicted FVC	2.2 (0.9 to 3.5) *	1.5 (-0.1 to 3.1)	2.6 (0.7 to 4.5) †	0.9 (0.5 to 1.4)	0.5 (-0.1 to 1.1)
FEV_1/FVC ratio	0.043 (0.034 to 0.053) *	0.041 (0.027 to 0.054)	0.045 (0.032 to 0.057)	0.036 (0.032 to 0.041)	0.029 (0.023 to 0.035)
% predicted $FEF_{25-75\%}$	14.2 (12.2 to 16.3)	14.7 (11.8 to 17.6)	14.0 (11.1 to 16.8)	13.8 (12.6 to 15.0) ¢	11.0 (8.9 to 13.0)
Baseline $\%FEV_1$ ≤85% predicted value	41 (38%) ¥¥, ***	15 (38%) ΔΔ	26 (38%) ≠≠, †††	100 (23%) ¢	31 (16%)
Positive BDR	20 (19%) ¥, ***	5 (13%)	15 (22%) ≠, †††	46 (11%) ¢	10 (6%)
FeNO	**n = 84**	**n = 34**	**n = 50**	**n = 390**	**n = 183**
FeNO >35ppb	6 (7%)	2 (6%)	4 (8%)	55 (14%)	20 (11%)

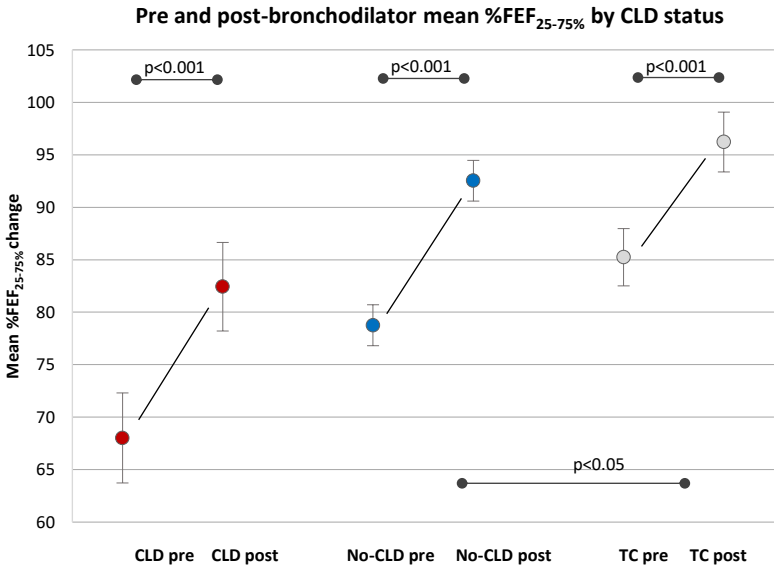
95% confidence intervals or percentages shown in brackets.

CLD vs No-CLD ¥ p<0.05, ¥¥ p<0.01, ¥¥¥ p<0.001 **CLD vs TC *p<0.05, ** p<0.01, *** p<0.001**
No-CLD vs TC ¢p<0.05, ¢¢p<0.01, ¢¢¢p<0.001 **CLD_{36} vs CLD_{28} ϟp<0.05, ϟϟp<0.01, ϟϟϟp<0.001,**
CLD_{36} vs No-CLD ≠p<0.05, ≠≠p<0.01, ≠≠≠p<0.001 **CLD_{36} vs TC †p<0.05, ††p<0.01, †††p<0.001**
CLD_{28} vs No-CLD Ψp<0.05, ΨΨp<0.01, ΨΨΨp<0.001 **CLD_{28} vs TC Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001**

Mean change in spirometry readings based on all children with pre- and post-BD spirometry.



a.



b.

Figure 3-4 Change in %FEV$_1$ and %FEF$_{25-75\%}$ following bronchodilator by CLD status.

Mean changes in spirometry and %FEV$_1$ and %FEF$_{25-75\%}$ with 95% CI in the CLD, No-CLD and TC groups. Significant differences in mean change within each group are detailed with p-values at the top of each chart. Significant differences in mean change between each group are detailed with p-values at the bottom of each chart.

a. Shows the mean %FEV$_1$ at baseline and post-bronchodilator for each group. All groups had significant changes in mean %FEV$_1$ post-bronchodilator. There were also significant differences between groups. Despite the CLD group having significantly higher mean %FEV$_1$ change compared to No-CLD and TC groups, the post-bronchodilator %FEV$_1$ remains below the baseline value observed in the TC group. Despite having a significantly lower baseline %FEV$_1$ compared with the TC group, post-bronchodilator, this difference is no longer significant in the No-CLD group.

b. Shows the mean %FEF$_{25-75\%}$ at baseline and post-bronchodilator for each group. Each group had significant changes in mean %FEF$_{25-75\%}$ post-bronchodilator. A significant difference in mean change in %FEF$_{25-75\%}$ between the No-CLD and TC groups was observed.

3.3.4 Incidence of lung function deficits

Subsequent to the observation of reduced mean baseline $\%FEV_1$ in both the No-CLD and CLD groups, further analysis of the proportions of children with clinically significant reduction in $\%FEV_1$ was undertaken. Using a $\%FEV_1$ value of ≤85% as a pragmatic proxy for LLN in children 7 to 12 years, I analysed the proportion of children with evidence of a $\%FEV_1$ ≤85% in all groups.

Both the CLD (38%) and No-CLD (23%) groups had higher incidence of a $\%FEV_1$ ≤85% when compared to the TC group (16%, $p<0.001$ and $p<0.05$ respectively), (Table 3-7). The CLD group had an increased OR for a $\%FEV_1$ ≤85% of 3.14 (95%CI 1.82 to 5.45) compared with the TC group and, 1.99 (95%CI 1.27 to 3.13) when compared with the No-CLD group. When compared with the TC group, the No-CLD group also had a significantly higher OR of 1.58 (95%CI 1.01 to 2.47) for a $\%FEV_1$ ≤85%.

3.3.5 Impact of CLD severity

By dividing the CLD group into those with CLD_{28} and CLD_{36} and comparing characteristics and lung function and with the No-CLD and TC groups, I assessed the impact that the severity of CLD has upon lung function.

3.3.5.1 Characteristics

Table 3-5 details the characteristics of children in the CLD_{28} and CLD_{36} groups. Whilst there were no significant differences between the CLD_{28} and CLD_{36} for gestation or birthweight, the CLD_{36} group had the largest proportion of those with IUGR compared with the CLD_{28} (24% vs 8%, $p<0.05$) and No-CLD (24% vs 13%, $p<0.05$) and TC (24% vs 5%, $p<0.001$) groups. The incidence of IUGR in the CLD_{28} group was similar to the TC group (8% vs 5%).

Disease severity was not associated with greater deprivation or proportions of children born after the Welsh smoking ban. The CLD_{36} group had the highest rates of maternal antenatal smoking compared with the CLD_{28} (18% vs 8%), No-CLD (18% vs 11%) groups. However, statistical difference was only found when comparing the CLD_{36} group to the TC group (18 vs 6%, $p<0.01$). The CLD_{28} group had comparable levels of maternal antenatal smoking with the TC group (8% vs 6%).

The CLD_{36} group also had the highest incidence of ROP, NEC and PDA when compared to the CLD_{28} group (ROP 25% vs 18%, NEC 25% vs 11%, PDA 35% vs 22%). These differences were not statistically significant; however, this may be due to the small number of children within

each group. Statistically significant rates of ROP, NEC and PDA were found when comparing the CLD_{36} with the No-CLD and TC groups. Similar differences were also observed when comparing the CLD_{28} group with the No-CLD and TC groups.

There were statistically significant differences between CLD_{28} and CLD_{36} groups in the proportions of children who required postnatal corticosteroids (5% vs 21% ,$p<0.05$), whilst home oxygen use was confined to 25 (37%) children in the CLD_{36} group ($p <0.001$).

The CLD_{36} group reported higher wheeze-ever (73% vs 59%) and wheeze in the last 12 months (32% vs 20%) compared to the CLD_{28} group. However, these differences were not statically significant. Both CLD_{36} and CLD_{28} groups had significantly higher wheeze-ever compared with the TC group (73% vs 27%, $p<0.001$ and 59% vs 27%, $p<0.001$ respectively). A significant difference in reported wheeze-ever was found between the CLD_{36} and No-CLD group (73% vs 50%, $p<0.01$) but not between CLD_{28} and No-CLD (59% vs 50%). The only significant difference observed in reported wheeze in the last 12 months was between the CLD_{36} and TC group (32% vs 13%, $p<0.01$). The CLD_{28} group reported higher incidence of asthma diagnosis and inhaler use, although this difference was only significant when compared with the TC group (Table 3-6).

3.3.5.2 Lung function

All baseline spirometry measures in the CLD_{36} group were significantly lower than the TC and No-CLD groups (Table 3-7). They were also lower than the CLD_{28} group but did not reach statistical significance. Baseline spirometry measures in the CLD_{28} group were also lower than the TC and No-CLD groups. However, only $\%FEV_1$ and $\%FEF_{25-75\%}$ between the CLD_{28} and TC groups were statistically different (88.7% vs 95.7%, $p<0.01$ and 73.6% vs 86.4%, $p<0.01$).

The proportions of children with a $\%FEV_1$ ≤85% in both the CLD_{36} and CLD_{28} were the same (38%). These proportions were lower than the No-CLD (23%) and TC group (16%).

3.3.5.3 BDR and FeNO

Despite post-bronchodilator increases, $\%FEV_1$, FEV_1/FVC and $FEF_{25-75\%}$ in the CLD_{36} group remained significantly lower than the No-CLD and TC groups. They also remained lower than the CLD_{28} group, but this difference was not statistically significant. Post-bronchodilator spirometry in the CLD_{28} group remained lower than both No-CLD and TC groups, but these differences were not significant.

The CLD_{36} group had the highest proportion of children (22%) with a positive BDR compared to CLD_{28} (13%), No-CLD (11%) and TC (6%) groups. The differences between the CLD_{36} and both the No-CLD and TC groups were statistically significant.

Despite these spirometry findings, proportions of those with a FeNO >35ppb in both CLD_{28} and CLD_{36} groups were lower (8% and 6% respectively) than the No-CLD (14%) and TC (6%) groups although these differences were not statistically different.

3.3.6 Summary of results

Children with a history of CLD were three times more likely than term-born controls and twice as likely as preterm-born children without CLD to have a $\%FEV_1$ ≤85%. Whilst severity of CLD did not impact the incidence of $\%FEV_1$ ≤85%, BDR or FeNO levels, those with CLD_{36} had the lowest spirometry measures and largest response to inhaled bronchodilator.

Preterm-born children without CLD were also one and a half times more likely to have a $\%FEV_1$ ≤85% than term-born controls. With 62% of children with CLD having normal lung function and 23% of the No-CLD group having a $\%FEV_1$ ≤85% it is reasonable to suggest that CLD may not be the optimal predictor of lung function deficits in the preterm-born population.

Children with CLD were born at earlier gestational age and lowest birthweight. They suffered poorer health during the neonatal period and reported greater incidence of respiratory illness in infancy compared to both the No-CLD and TC groups. However, with similar incidence of current reported respiratory symptoms between those with and without CLD, it may be reasonable to suggest that a diagnosis of CLD may reflect poor respiratory health that is limited to early life.

Despite 38% of the CLD and 23% of the No-CLD groups having a $\%FEV_1$ ≤85%, a lower proportion of both groups (19% and 11% respectively) had a positive BDR suggesting some children have degree of irreversible or fixed airflow limitation. The similar proportions of children with FeNO >35ppb across all groups suggests Th2 driven eosinophilic inflammation is not associated with CLD status.

There was, therefore, a need to further analyse of the whole preterm-born population by current $\%FEV_1$ to improve understanding of the predictive value of CLD and other early life factors including sex, gestation, IUGR, deprivation and maternal smoking on lung function in childhood.

3.4 Early life factors associated with lung function deficits in the preterm-born population

Having demonstrated that a proportion of preterm-born school-aged children with and without a history of CLD have ongoing lung function deficits, this section aimed to clearly define the lung function deficits in the preterm-born population based on current spirometry and explore early life factors associated with lung function deficits.

Analysis of demographic details and lung function measures were undertaken by dividing the preterm-born population into those with a $\%FEV_1 \leq 85\%$ labelled P_{low} and those with a $\%FEV_1 > 85\%$ who formed a preterm-born control group (PTC) and comparing with term-born controls (TC). Assessments of BDR and FeNO in the P_{low}, PTC and TC groups are described. Exploration of early life factors that may help to identify those at higher risk of lung function impairment was also undertaken.

3.4.1 Characteristics of participants by current $\%FEV_1$

Table 3-8 describes the characteristics of all three groups. In total, 141 (26%) of all preterm-born participants had a $\%FEV_1 \leq 85\%$. The P_{low} and PTC groups had a lower WIMD scores (Mean (SD), P_{low} 1064 (577) and PTC 1064 (569)) than the TC group (Mean (SD), 1176 (524)), but these differences were not statistically different. A significantly lower proportion of children in the P_{low} group compared with both PTC and TC were born after the introduction of the Welsh smoking ban 2007 (58% vs 72% $p<0.001$, and 58% vs 79% $p<0.001$, respectively). The TC group had significantly lower rates of maternal antenatal and postnatal smoking compared to both the P_{low} and PTC groups. However, there were no differences in the rates of maternal antenatal and postnatal smoking between the P_{low} and PTC groups.

As expected, both preterm-born groups were born at lower gestations and birthweight than term-born controls. They also had greater morbidity in the neonatal period and reported greater respiratory disease in infancy. Acknowledging that these findings are consistent with earlier comparisons of characteristics between the preterm- and the term-born groups, discussion related to participant characteristics focuses on the differences between the two preterm-born groups.

The P_{low} group had a lower median gestation at birth than the PTC group (31 vs 32 weeks', $p<0.01$), lower birth weight (1450gms vs 1758gms, $p< 0.01$) and higher incidence of IUGR (20% vs 12%, $p<0.001$). The incidence of PROM, birth by LSCS, use of maternal antenatal

corticosteroid administration and family history of atopy were comparable between the two preterm-born groups.

Compared with the PTC group, the P_{low} group had higher incidence of IVH (18% vs 7%, $p<0.001$), chest drain insertion (9% vs 2%, $p<0.01$) and combined illness (30% vs 14%, $p<0.001$). Despite the P_{low} group having consistently higher proportions of those with home oxygen, ROP, NEC and PDA compared to the PTC group, they were not significant. The incidence of CLD was also higher in the P_{low} group (29% vs 16%, $p<0.01$), with a larger proportion of those with CLD_{36} in the P_{low} group (18% vs 10%, $p<0.05$). However, it is notable that 16% of those in the PTC group also had CLD.

Table 3-8 Characteristics of participants based on %FEV_1

	≤34/40 % predicted FEV_1 ≤85 (P_{low})	≤34/40 control % predicted FEV_1 >85 (PTC)	≥37/40 Term-born controls (TC)
Subjects (n)	141 (26%) [b]	403 (74%) [b]	195 (26%) [a]
Current status			
Male	68/141 (52%)	211/403 (52%)	100/195 (51%)
Current Age (y)	9.9 (9.7 to 10.2) ∞∞∞	9.5 (9.4 to 9.7)	9.7 (9.5 to 9.8)
Current Height (cm)	140.7 (139.1 to 142.4)∞∞	139.5 (138.4 to 140.5) ×	142.0 (140.7 to 143.3)
Current Weight (kg) (range)	32.4 (18.2 to 62.8)	32.8 (17.7 to 88.9) ×	34.8 (21.4 to 78.2)
Current BMI (range)	16.5 (12.2 to 28.0)	17.0 (12.8 to 32.5)	17.1 (13.2 to 30.9)
WIMD 2019 (Mean SD)	1064 (577)	1064 (569)	1176 (524)
Born post-smoking ban (2007)	82/141 (58%)∞∞∞∞, ΘΘΘ	291/403 (72%)	153/195 (79%)
Neonatal History			
Gestational Age (wks) (range)	31 (24 to 34) ∞∞∞, ΘΘΘ	32 (23 to 34) ×××	40 (37 to 42)
Birth weight (g) (range)	1450 (482 to 2930) ∞∞∞, ΘΘΘ	1758 (450 to 3912) ×××	3430 (2155 to 4916)
Birthweight adjusted z-score	-0.087 (-0.312 to 0.139) ∞∞	0.256 (0.124 to 0.387)	0.062 (-0.028 to 0.199)
IUGR	28/141 (20%) ∞∞, ΘΘΘ	48/403 (12%) ××	9/195 (5%)
PROM	51/137 (37%) ΘΘΘ	150/393 (38%) ×××	6/185 (3%)
C/S delivery	79/141 (56%) ΘΘΘ	227/401 (57%) ×××	49/195 (25%)
Antenatal corticosteroids	122/135 (90%) ΘΘΘ	329/376 (88%) ×××	4/195 (2%)
Postnatal corticosteroids	6/131 (5%) ΘΘ	8/383 (2%) ×	0/194 (0%)
CLD	41/141 (29%) ∞∞∞	67/403 (16%)	0/195
CLD_{28}	15/141 (11%)	25/403 (6%)	N/A
CLD_{36}	26/141 (18%) ∞∞,	42/403 (10%)	N/A
Home oxygen	9/138 (7%) ΘΘΘ	16/397 (4%) ××	0/194 (0%)
ROP	13/141 (9%) ΘΘΘ	19/403 (5%) ×××	0/195 (0%)
Chest drain	12/138 (9%) ∞∞∞, ΘΘΘ	7/392 (2%)	0/193 (0%)
IVH	26/141 (18%) ∞∞∞∞, ΘΘΘ	30/403 (7%) ×××	0/195 (0%)
NEC	13/137 (10%) ΘΘΘ	18/390 (4%) ××	0/194 (0%)
PDA	14/138 (10%) ΘΘΘ	21/388 (5%) ×××	0/195 (0%)
Combined Illness	41/139 (30%) ∞∞∞∞, ΘΘΘ	53/391 (14%) ×××	0/194 (0%)
Family History			
Maternal antenatal smoking	14/138 (10%)	48/394 (12%) ××	11/194 (6%)
Maternal postnatal smoking	16/140 (11%) Θ	58/399 (15%) ×××	8/195 (4%)
F/H Asthma	83/140 (59%) Θ	213/399 (53%)	91/195 (47%)
F/H Hay fever	72/138 (52%)	217/396 (55%)	115/194 (59%)
F/H Eczema	59/138 (43%)	183/393 (47%)	90/195 (46%)
F/H Allergies	52/138 (38%)	156/393 (40%)	81/193 (42%)

95% confidence intervals or percentages shown in brackets unless ranges specified. [a] = % of total population, [b] = % preterm-born population. **P_{low} v PTC ∞p<0.05, ∞∞p<0.01, ∞∞∞∞p<0.001, P_{low} v TC Θp,0.05, ΘΘp<0.01, ΘΘΘp<0.001, PTC v TC ×p<0.05, ××p<0.01, ×××p<0.001.** Adjusted birthweight z-score - adjustments for gestation and sex. *Abbreviations: PROM = prolonged rupture of membranes, C/S = caesarean section delivery, CLD = Chronic lung disease, ROP = retinopathy of prematurity, NEC = necrotising enterocolitis requiring treatment, PDA = patent ductus arteriosus requiring medical or surgical treatment, Combined Illness = IVH or ROP or NEC in the neonatal period, Chest drain = insertion to treat pneumothorax, FH = family history.*

Comparison of respiratory illness and symptoms between P_{low} and PTC detailed in Table 3-9 showed that the P_{low} group reported higher incidence of bronchiolitis (28% vs 15%, p<0.01), viral-induced wheeze (16% vs 8%, p<0.01), wheeze-ever (63% vs 50%, p<0.05), inhaler use (23% vs 15%, p<0.05) and diagnosis of asthma (25% vs 13%, p<0.01). Reports of wheeze in the last 12 months were also higher, but not statistically significant (34% vs 26%, p 0.179).

Table 3-9 Postnatal respiratory health by %FEV_1 status

	≤34/40 % predicted FEV_1 ≤85 (P_{low})	**≤34/40 control % predicted FEV_1 >85 (PTC)**	**≥37/40 Term-born controls (TC)**
Subjects (n)	141 (26%) [b]	403 (74%) [b]	195 (26%) [a]
Bronchiolitis	39/141 (28%) ∞∞, ΘΘ	58/401 (15%) ××	10/194 (5%)
Viral-induced wheeze	22/141 (16%) ∞∞, ΘΘ	30/400 (8%)	7/195 (4%)
Pneumonia	13/141 (9%) Θ	21/401 (5%)	6/194 (3%)
Wheeze-ever	87/138 (63%) ∞, ΘΘ	194/385 (50%)	51/191 (27%)
Wheeze last 12 months	48/141 (34%) ΘΘ	104/403 (26%) ××	25/195 (13%)
Inhalers last 12 months	32/141 (23%) ∞,ΘΘ	61/403 (15%) ××	12/195 (6%)
Diagnosed Asthma	35/140 (25%) ∞∞, ΘΘ	53/403 (13%) ××	10/193 (5%)

[a] = % of total population, [b] = % preterm-born population.

P_{low} v PTC ∞p<0.05, ∞∞p<0.01, ∞∞∞∞p<0.001 P_{low} v TC Θp,0.05, ΘΘp<0.01, ΘΘΘp<0.001 PTC v TC ×p<0.05, ××p<0.01, ×××p<0.001

3.4.2 Lung function by current %FEV_1

From 544 preterm-born children who completed baseline spirometry, 141 preterm-born children had a %FEV_1 ≤85% (P_{low}) and 403 had a %FEV_1 >85% (PTC). Data from the 195 term-born controls (TC) were also included. Both the P_{low} and PTC groups had 5% of children who did not perform satisfactory post-bronchodilator spirometry. Similarly, 6% of the TC group did not complete post-bronchodilator spirometry. The proportions of children unable to perform FeNO in the P_{low} and PTC groups were also similar with rates of 12% and 13% respectively. Six percent of the TC group could not perform FeNO.

Unsurprisingly, the selection of preterm-born children by %FEV_1 status resulted in the P_{low} group having significantly lower measures across all baseline spirometry compared to both the PTC and TC groups. The P_{low} group had significantly lower baseline %FEV_1 compared to both the PTC and TC groups (75.6% vs 96.6%, p<0.001 and 75.6% vs 95.7%, p<0.001 respectively) and significantly lower %FVC compared to the PTC and TC groups (83.2% vs 98.2%, p<0.001 and 83.2% vs 96.2%, p<0.001 respectively). Similarly, the P_{low} group had significantly lower FEV_1/FVC compared with the PTC and TC groups (0.80 vs 0.86, p<0.001 and 0.80 vs 0.87, p<0.001 respectively) and lower $FEF_{25-75\%}$ compared with the PTC and TC groups (57% vs 84%, p<0.01 and 57% vs 86.4%, p<0.01 respectively). In contrast, there were no differences in baseline spirometry when comparing the PTC group with the TC group. (Table 3-10).

3.4.3 BDR and FeNO

Post-bronchodilator, the P_{low} group had the largest mean increase in %FEV_1 from 75.6% to 83.3% (increase 7.9%, 95%CI 6.5 to 9.3%) (Table 3-10). They also had the largest increase in mean %FVC from 83.2% to 86.4% (increase 3.0%, 95%CI 1.8 to 4.2%), FEV_1/FVC from 0.80 to 0.85 (mean change 0.054, 95%CI 0.044 to 0.064) and increase in %$FEF_{25-75\%}$ from 57.0% to 71.8% (mean change 15.2%, 95%CI 13.6 to 17.4). These mean increases in %FEV_1, %FVC, FEV_1/FVC observed in the P_{low} group were all highly significant (p<0.001) when compared with both the PTC and TC groups. Whilst comparison of mean change in %$FEF_{25-75\%}$ between the P_{low} and PTC groups was not significant (p 0.506), it was significantly different when comparing the P_{low} with the TC group (p<0.05).

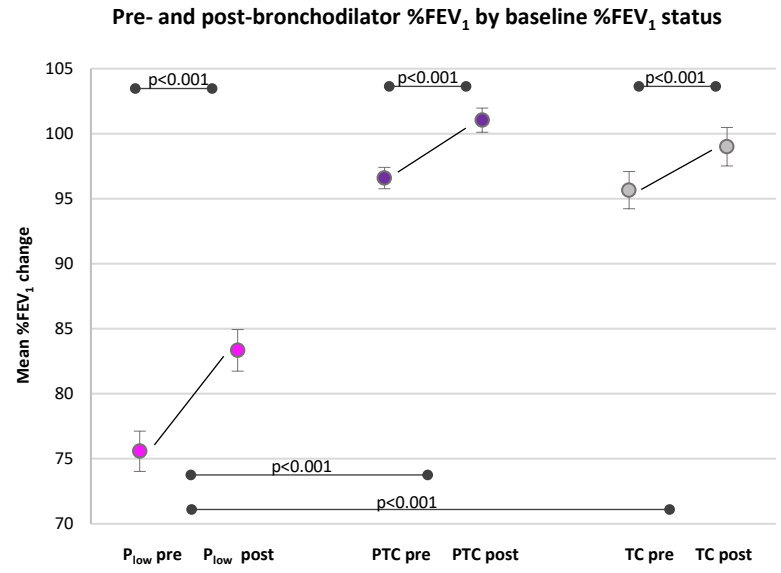
Despite the P_{low} group having the greatest increases in spirometry measures post-bronchodilator, the group continued to have significantly lower measures than both the PTC and TC groups (Figure 3-5).

Compared with PTC and TC groups, the P_{low} group had the highest proportion of children with a positive BDR (29% vs 7%, $p<0.001$ and 29% vs 6%, $p<0.001$ respectively) and FeNO >35ppb (23% vs 9%, $p<0.001$ and 23% vs 11%, $p<0.01$ respectively). The were no differences between the proportions of the PTC and TC groups with a positive BDR or FeNO >35ppb.

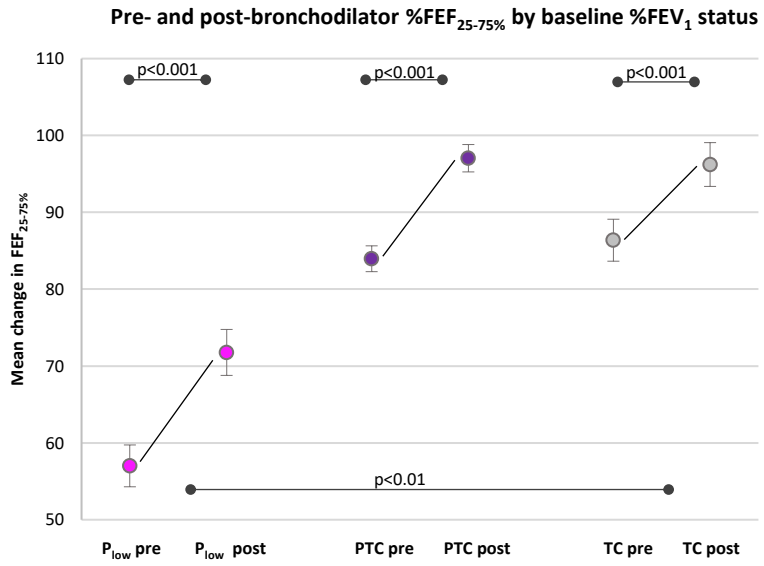
Table 3-10 Lung function by %FEV_1 status

	≤34/40 % predicted FEV_1 ≤85 (P_{low})	≤34/40 control % predicted FEV_1 >85 (PTC)	≥37/40 Term-born control (TC)
Baseline spirometry	**n = 141**	**n = 403**	**n = 195**
% predicted FEV_1	75.6 (74.0 to 77.1) ΘΘ,∞∞∞∞	96.6 (95.8 to 97.4)	95.7 (94.2 to 97.0)
% predicted FVC	83.2 (81.8 to 84.6) ΘΘ,∞∞∞∞	98.2 (97.2 to 99.1)	96.2 (94.8 to 97.7)
FEV_1/FVC ratio	0.80 (0.78 to 0.81) ΘΘ,∞∞∞∞	0.86 (0.86 to 0.87)	0.87 (0.86 to 0.88)
% predicted $FEF_{25-75\%}$	57.0 (54.2 to 59.7) ΘΘ,∞∞∞∞	84.0 (82.3 to 85.6)	86.4 (83.6 to 89.1)
Post-BD spirometry	**n = 134**	**n = 382**	**n = 183**
% predicted FEV_1	83.3 (81.8 to 84.9) ΘΘ,∞∞∞∞	101.0 (100.1 to 102.0)	99.0 (97.5 to 100.5)
% predicted FVC	86.4 (84.7 to 88.2) ΘΘ,∞∞∞∞	99.0 (98.0 to 100.0) ×	96.7 (95.2 to 98.3)
FEV_1/FVC ratio	0.85 (0.83 to 0.86) ΘΘ,∞∞∞∞	0.89 (0.89 to 0.90)	0.89 (0.89 to 0.90)
% predicted $FEF_{25-75\%}$	71.8 (68.8 to 74.8) ΘΘ,∞∞∞∞	97.0 (95.3 to 98.8)	96.2 (93.4 to 99.1)
Mean Change in spirometry ##	**n = 134**	**n = 382**	**n = 183**
% predicted FEV_1	7.9 (6.5 to 9.3) ΘΘΘ,∞∞∞∞	4.4 (3.9 to 4.8)	3.6 (2.9 to 4.3)
% predicted FVC	3.0 (1.8 to 4.2) ΘΘΘ,∞∞∞∞	0.6 (0.1 to 1.0)	0.5 (-0.1 to 1.1)
FEV_1/FVC ratio	0.054 (0.044 to 0.064) ΘΘΘ,∞∞∞∞	0.032 (0.028 to 0.036)	0.029 (0.023 to 0.035)
% predicted $FEF_{25-75\%}$	15.2 (13.6 to 17.4) ΘΘ	13.3 (12.1 to 14.6)	11.0 (8.9 to 13.0)
Baseline %FEV_1 ≤85% predicted value	141 (100%)	0 (0%)	31 (16%)
Positive BDR	39 (29%) ΘΘΘ,∞∞∞∞	27 (7%)	10 (6%)
FeNO	**n = 124**	**n = 350**	**n = 183**
FeNO >35ppb	28 (23%) ΘΘ,∞∞∞∞	33 (9%)	20 (11%)

95% confidence intervals or percentages shown in brackets. ## Mean change in spirometry readings based on all children with pre- and post-BD spirometry. **PTC v TC ×p<0.05, ××p<0.01, ×××p<0.001 P_{low} v PTC ∞p<0.05, ∞∞p<0.01, ∞∞∞∞p<0.001 P_{low} v TC Θp,0.05, ΘΘp<0.01, ΘΘΘp<0.001**



a.



b.

Figure 3-5 Change in $\%FEV_1$ and $\%FEF_{25-75\%}$ following bronchodilator by baseline $\%FEV_1$ status.

Mean changes in spirometry and $\%FEV_1$ and $\%FEF_{25-75\%}$ with 95% CI in the P_{low}, PTC and TC groups. Significant differences in mean change within each group are detailed with p-values at the top of each chart. Significant differences in mean change between each group are detailed with p-values at the bottom of each chart.

a. Shows mean $\%FEV_1$ at baseline and post-bronchodilator for each group. All groups had significant changes in mean $\%FEV_1$ post-bronchodilator. The P_{low} group had lower mean $\%FEV_1$ at baseline and had a significantly larger mean change in $\%FEV_1$ compared to both PTC and TC groups. Despite this increase, the mean $\%FEV_1$ post-bronchodilator remained lower than both control groups. Mean $\%FEV_1$ at baseline and post-bronchodilator were comparable between PTC and TC groups.

b. Shows mean $\%FEF_{25-75\%}$ at baseline and post-bronchodilator in all groups. All groups had significant changes in mean $\%FEF_{25-75\%}$ post-bronchodilator. The P_{low} group had lowest baseline $\%FEF_{25-75\%}$. They had a significantly larger mean change in $\%FEF_{25-75\%}$ compared to TC only. Despite the increase in $\%FEF_{25-75\%}$ in the P_{low} group it remained significantly lower than PTC and TC groups. Mean $\%FEF_{25-75\%}$ was comparable between PTC and TC groups at baseline and post-bronchodilator.

3.4.4 Early life factors that influence low %FEV_1 in childhood

With higher proportions of children with and without a history of CLD in infancy currently having a %FEV_1 ≤85% compared to term-born controls (38%, 23% and 16% respectively), the value of CLD as a predictor for childhood lung function deficits becomes questionable. Therefore, modelling was undertaken to identify covariables which may help identify preterm-born children at risk for childhood lung function deficits.

Using the baseline %FEV_1 ≤85%, or not as a binary outcome, univariable logistic regression analysis in the whole preterm-born population detailed in Table 3-11 was undertaken. Demographic covariables and factors identified as significant on prior analysis of the preterm-born population by CLD status and baseline %FEV_1 in this study were included in the initial explorative modelling. Sex - a factor previously known to impact on respiratory outcomes in this population - was also included in this initial analysis.

This regression analysis identified an inverse relationship with gestational age (Beta -0.153, p<0.001) alongside a significant association with CLD (all cases) (OR 2.06, 95%CI 1.31 to 3.22), IUGR (OR 1.83, 95%CI 1.1 to 3.06) and CLD_{36} (OR 1.94, 95%CI 1.14 to 3.31). Interestingly, whilst there was a significant association between baseline %FEV_1 and CLD_{36}, there was no significant association with CLD_{28}. Other factors for which there was no significant association with baseline %FEV_1 were deprivation, family history of asthma, sex, maternal antenatal and postnatal smoking, PROM, and birth by caesarean section.

Table 3-11 Univariable analysis of predictors of a %FEV_1 ≤85% in the preterm-born population

Participants n= 544			
Covariables	**Beta**	**Standard error**	**Significance**
Gestational age	**-0.153**	**0.036**	**0.000**
Factors	**Beta**	**Odds ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	0.606	**1.83 (1.10, 3.06)**	**0.020**
CLD (ref = No)	0.721	**2.06 (1.31, 3.22)**	**0.002**
CLD_{36} (ref = No)	0.664	**1.94 (1.14, 3.31)**	**0.014**
CLD_{28} (ref = No)	0.588	1.80 (0.92, 3.52)	0.086
FH asthma* (ref = No)	0.240	1.27 (0.86, 1.88)	0.228
Sex (ref = Female)	-0.165	0.85 (0.58, 1.24)	0.399
AN maternal smoking* (ref = No)	-0.206	0.81 (0.43, 1.53)	0.521
PN maternal smoking* (ref = No)	-0.276	0.76 (0.42 to 1.37	0.359
LSCS* (ref = No)	-0.024	0.98 (0.66 to 1.44)	0.905
WIMD Quintiles			
1 – Most deprived	-0.110	0.99 (0.54 to 1.80)	0.971
2	-0.181	0.84 (0.46 to 1.51)	0.550
3	0.242	1.27 (0.73 to 2.23)	0.397
4	-0.184	0.83 (0.47 to 1.48)	0.532
5 – Least deprived	Ref	-	-

Covariables presented as Beta value and SE. Beta value and Odds ratios with (95%CI) presented for all factors.
*Missing cases: Family history of asthma = 5, Antenatal smoking = 12, Postnatal smoking = 5, Caesarean section = 2. *Abbreviation: WIMD: Welsh Index of Multiple Deprivation 2019.*

Early and current life factors identified as having a p<0.1 during the univariable logistic regression were included in multivariable logistic regression.

Two models were developed, both with the binary outcome of baseline %FEV_1 ≤85%, or not. The first model used CLD (all cases) as a binary categorical factor and the second used the more specific CLD_{36} as a binary categorical factor. Details of both models are seen in Table 3-12 and Table 3-13.

Model 1 showed that whilst gestation (Beta -0.153, p 0.003) and IUGR remained significantly associated with a %FEV_1 ≤85% (OR 1.78, 95%CI 1.06 to 3.00), CLD (all cases) did not (OR 0.99, 95%CI 0.52 to 1.89). Model 2 also showed that gestation (Beta -0.161, p0.003) and IUGR (OR 1.78 95%CI 1.06 to 3.00) were significantly associated with a %FEV_1 ≤85%, but CLD_{36} was not (OR 0.88, 95%CI 0.45 to 1.72).

Table 3-12 Multivariable modelling for a %FEV_1 ≤85% in the preterm-born population. All CLD cases.

Model 1			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	**-0.153**	**0.051**	**0.003**
Factors	**Beta**	**Odds Ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	**0.579**	**1.78 (1.06, 3.00)**	**0.029**
CLD (ref = No)	-0.11	0.99 (0.52, 1.89)	0.974
Covariables presented as Beta value and SE. Beta value and Odds Ratios with 95%CI presented for all factors.			

Table 3-13 Multivariable modelling for a %FEV_1 ≤85% in the preterm-born population. Cases of CLD_{36}.

Model 2			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	**-0.161**	**0.044**	**<0.000**
Factors	**Beta**	**Odds Ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	**0.588**	**1.80 (1.07, 3.04)**	**0.028**
CLD_{36} (ref = No)	-0.124	0.88 (0.45, 1.72)	0.716
Covariables presented as Beta value and SE. Beta value and Odds Ratios with (95%CI) presented for all factors.			

3.4.5 Summary of results

Preterm-born children with a current %FEV_1 ≤85% showed evidence of significant lung function deficits when compared to preterm- and term-born controls who had comparable lung function measures. A greater proportion of children with a %FEV_1 ≤85% had evidence of ongoing Th2 driven eosinophilic inflammation and were 7 times more likely to have a positive BDR compared to term-born controls and 5 times more likely when compared with preterm-born controls.

Exploratory analysis of significant early life factors associated with lung function deficits in childhood suggested that the predictive value of CLD is limited whilst gestational age at birth and IUGR may be superior predictors of a %FEV_1 ≤85% in childhood.

Despite the %FEV_1 identifying those with lung function deficits, only 29% of these children had a positive BDR and 23% had evidence of a FeNO >35ppb. This suggests there may be distinct respiratory phenotypes within this population, who have different underlying lung pathologies. Identification of different respiratory phenotypes may help identify those who should respond to currently available treatments. Further exploration into the phenotypes of lung function deficits in the preterm-born population and response to inhaled treatments was undertaken.

3.5 Defining respiratory phenotypes in preterm-born children with lung function deficits

Analysis of $\%FEV_1$ in preterm-born school-aged children has provided a clear delineation between those with and without lung function deficits, with preterm-born children with $\%FEV_1$>85% and term-born controls having comparable spirometry, response to bronchodilator and similar proportions with FeNO >35ppb.

I further divided those with lung function deficits into obstructive and non-obstructive respiratory phenotypes. In the absence of a TLC measurement, I used the LLN for FEV_1/FVC ratio in males and females between 7 and 12 years of age as a proxy measurement that could be easily applied to all participants in my study to identify those with obstructive and non-obstructive respiratory phenotypes (Table 3-14). Consequently, preterm-born children with a $\%FEV_1 \leq 85\%$ and FEV_1/FVC ratio <0.8 were classified as obstructive and those with baseline $\%FEV_1 \leq 85\%$ and FEV_1/FVC ratio ≥0.8 were classified as non-obstructive. Despite the NICE guidelines suggesting FEV_1/FVC of 0.7 as the cut-off to identify children with obstructive disease, this recommendation is not based on robust evidence (NICE 2017a). To my knowledge this is the first occasion that this strategy has been used.

Table 3-14 FEV_1/FVC Lower Limits of Normal in males and females by age

Age	Male LLN FEV_1/FVC	Female LLN FEV_1/FVC
7	0.78	0.8
8	0.77	0.79
9	0.76	0.79
10	0.76	0.78
11	0.75	0.78
12	0.75	0.78

LLN calculated from GLI reference ranges. Whole ages, Caucasian ethnicity and heights based on 50th percentile from UK-WHO growth charts for males and females were used for calculation purposes.

Despite questioning the value of CLD as a predictor for reduced %FEV_1, there is evidence that CLD is associated with airflow obstruction (Doyle et al. 2006; Kotecha et al. 2013). It was therefore decided to examine whether CLD has a role in different phenotypes in those with a low %FEV_1. I hypothesised that those with obstructive patterns of lung function deficits would have a greater response to inhaled bronchodilator and that children with a history of CLD have higher incidence of obstructive lung function deficits.

3.5.1 Characteristics of children with obstructive and non-obstructive respiratory phenotypes

A total of 141 preterm-born children had a %FEV_1 ≤85% and were included in the analysis of different respiratory phenotypes. Fifty-eight children (41%) were classified as obstructive ($P_{low(O)}$) and 83 (59%) as non-obstructive ($P_{low(NO)}$).

A higher proportion of the $P_{low(O)}$ group had a history of CLD (40% vs 22%, $p<0.05$) and NEC (16% vs 5%, $p<0.05$) compared to the $P_{low(NO)}$ group. There were no other significant differences in neonatal characteristics between those in the $P_{low(O)}$ and $P_{low(NO)}$ groups (Table 3-15).

There were significant differences in the occurrence of respiratory illness in infancy/childhood, current symptoms, and treatment between the two groups. Those in the $P_{low(O)}$ group reported higher incidence of viral-induced wheeze (24% vs 10%, $p<0.05$), wheeze-ever (79% vs 52%, $p<0.01$), wheeze in the last 12 months (52% vs 22%, $p<0.001$), diagnosis of asthma (39% vs 16%, $p<0.01$) and inhaler use (40% vs 11%, $p<0.001$) compared to the $P_{low(NO)}$ group (Table 3-16)

Irrespective of whether the observed lung function deficits were obstructive or non-obstructive, those in the CLD groups were born earlier and smaller and had greater neonatal illness than the No-CLD groups. Whilst a higher proportion of children without CLD in the non-obstructive phenotype (No-$CLD_{low(NO)}$) had IUGR (19%) compared to those with CLD in the non-obstructive phenotype ($CLD_{low(NO)}$) (6%), this finding was not statistically significant (Table 3-15).

Respiratory symptoms and illness were similar between the obstructive and non-obstructive respiratory phenotypes irrespective of CLD status. The obstructive groups in both those with CLD ($CLD_{low(O)}$) and without CLD (No-$CLD_{low(O)}$) had greater illness and respiratory symptoms compared to the non-obstructive groups.

Despite similar incidence of respiratory illness and symptoms, fewer of the $CLD_{low(O)}$ group had been prescribed inhalers compared to those in the No-$CLD_{low(O)}$ group (22% vs 51%, $p<0.05$). There were also fewer children in the $CLD_{low(O)}$ group who had been subsequently diagnosed with asthma compared to those in the No-$CLD_{low(O)}$ group, although this difference was not significant (Table 3-16).

CLD did not have a dominant impact on the characteristics of either obstructive or non-obstructive groups. However, a higher proportion of those with CLD (56%) had an obstructive pattern of lung function deficits compared to those with No-CLD (35%). Children with a low $\%FEV_1$ who had a history of CLD had an OR of 2.37 (95% CI 1.13 to 4.98) of developing obstructive lung function deficits compared to those who did not have CLD (Figure 3-6).

Table 3-15 Characteristics of preterm-born children with obstructive and non-obstructive respiratory phenotypes

	P_{low} n = 141		Preterm-born population with % predicted FEV_1 ≤85% (P_{low}) n = 141.			
			CLD_{low} n = 41		No-CLD_{low} n = 100	
	$P_{low(NO)}$ FEV_1/FVC ratio <0.8 (Non-obstructive)	$P_{low(O)}$ FEV_1/FVC ratio <0.8 (Obstructive)	$CLD_{low(NO)}$ FEV_1/FVC ratio <0.8 (Non-obstructive)	$CLD_{low(O)}$ FEV_1/FVC ratio <0.8 (Obstructive)	No-$CLD_{low(NO)}$ FEV_1/FVC ratio <0.8 (Non-obstructive)	No-$CLD_{low(O)}$ FEV_1/FVC ratio <0.8 (Obstructive)
Subjects (n)	83 (59%) [a]	58 (41%) [a]	18 (44%) [b]	23 (56%) [b]	65 (65%) [c]	35 (35%) [c]
Male	37/83 (45%)	27/58 (47%)	9/18 (50%)	11/23 (48%)	28/65 (43%)	20/35 (57%)
Current Age (y) (95% CI)	9.9 (9.6 to 10.2)	10.0 (9.6 to 10.3)	10.3 (9.7 to 11.0)	10.1 (9.5 to 10.7)	9.8 (9.5 to 10.2)	9.9 (9.4 to 10.3)
Current Height (95% CI)	141.3 (139.1 to 143.5)	139.9 (137.5 to 142.4)	142.8 (137.6to 147.9)	139.3 (134.7 to 143.8)	140.9 (138.3 to 143.4)	140.4 (137.4 to 143.4)
Current Weight (kg) (range)	32.3 (18.2 to 62.8)	32.5 (19.4 to 61.3)	35.8 (20.4 to 56.1)	34.1 (19.4 to 58.3)	31.6 (18.2 to 62.8)	32.4 (21.4 to 61.3)
Current BMI (range)	16.2 (12.2 to 26.3)	17.0 (13.3 to 28.0)	16.8 (13.3 to 26.3)	17.6 (13.9 to 24.3)	16.0 (12.2 to 25.8)	16.6 (13.3 to 28.0)
Neonatal History						
Gestational Age (wks) (range)#	31 (24 to 34)	30 (24 to 34)	26 (24 to 31) ʒʒʒ	27 (24 to 31) ɯɯɯ	31 (26 to 34)	32 (27 to 34)
Birth weight (g) (range)#	1503 (500 to 2930)	1333 (482 to 2608)	906 (710 to 1502) ʒʒʒ	910 (482 to 2300) ɯɯɯ	1701 (500 to 2930)	1842 (850 to 2608)
Birthweight Adj.Z-score (95% CI)	0.053 (-0.250 to 0.355)	-0.286 (-0.627 to 0.054)	-0.006 (-0.468 to 0.480)	-0.179 (-0.760 to 0.403)	0.066 (-0.304 to 0.435)	-0.357 (-0.795 to 0.080)
IUGR	13/83 (16%)	15/58 (26%)	1/18 (6%)	5/23 (22%)	12/65 (19%)	10/35 (29%)
PROM	26/81 (32%)	25/56 (45%)	4/18 (22%)	10/23 (44%)	22/63 (35%)	15/33 (46%)
C/S delivery	51/83 (61%)	28/58 (48%)	8/18 (44%)	11/23 (48%)	43/65 (66%)	17/35 (49%)
A/N corticosteroids	72/81 (89%)	50/54 (93%)	16/18 (89%)	23/23 (100%)	56/63 (89%)	27/31 (87%)
P/N corticosteroids	3/79 (4%)	3/52 (6%)	3/18 (17%) ʒ	3/18 (17%) ɯ	0/61 (0%)	0/34 (0%)
CLD	18/83 (22%) §	23/58 (40%)				
CLD28	8/83 (10%)	7/58 (12%)	8/18 (44%)	7/23 (30%)		
CLD36	10/83 (12%)	16/58 (28%)	10/18 (56%)	16/23 (70%)		
Home oxygen	3/82 (4%)	6/56 (11%)	3/18 (17%) ʒʒ	6/22 (27%) ɯɯ	0/64 (0%)	0/34 (0%)
ROP	8/83 (10%)	5/58 (9%)	5/18 (28%) ʒ	4/23 (17%)	3/65 (5%)	1/35 (3%)
Chest drain	6/83 (7%)	6/55 (11%)	1/18 (6%)	2/22 (9%)	5/65 (8%)	4/33 (12%)
IVH	17/83 (21%)	9/58 (16%)	7/18 (39%) ʒ	6/23 (26%)	10/65 (15%)	3/35 (9%)
NEC	4/80 (5%) §	9/57 (16%)	2/17 (12%)	6/23 (26%)	2/63 (3%)	3/34 (9%)
PDA	7/82 (9%)	7/56 (13%)	6/18 (33%) ʒʒʒ	6/23 (26%) ɯ	1/64 (2%)	1/33 (3%)
Combined Illness	22/82 (27%)	19/57 (33%)	10/18 (56%) ʒʒ	13/23 (57%) ɯɯ	12/64 (19%)	6/34 (18%)

[a] % of preterm-born with low lung function, [b] % of preterm-born with CLD and low lung function, [c] % of preterm-born without CLD and low lung function. Results are expressed as mean (95% CI), unless specified.

$P_{low(NO)}$ v $P_{low(O)}$ §p<0.05, §§p<0.01, §§§p<0.001 $CLD_{low(NO)}$ v $CLD_{low(O)}$ ℓp<0.05, ℓℓp<0.01, ℓℓℓp<0.001 No-$CLD_{low(NO)}$ v No-$CLD_{low(O)}$ ¥p<0.05, ¥¥p<0.01, ¥¥¥p<0.001. $CLD_{low(NO)}$ v No-$CLD_{low(NO)}$ ʒp<0.05, ʒʒp<0.01, ʒʒʒp<0.001 $CLD_{low(O)}$ v No-$CLD_{low(O)}$ Ɯp<0.05, ƜƜp<0.05, ƜƜƜp<0.001

Abbreviations: IUGR = intrauterine growth restriction, PROM = prolonged rupture of membranes, C/S = caesarean section delivery, CLD = Chronic lung disease, ROP = retinopathy of prematurity, NEC = necrotising enterocolitis requiring treatment, PDA = patent ductus arteriosus requiring medical or surgical treatment, Combined Illness = IVH or ROP or NEC in the neonatal period, Chest drain = insertion to treat pneumothorax, A/N = antenatal and P/N = postnatal, FH = family history.

Table 3-16 Family history and respiratory symptoms in preterm-born children with obstructive and non-obstructive respiratory phenotypes

	P_{low} n = 141		Preterm-born population with % predicted FEV_1 ≤85% (P_{low}) n = 141.			
			CLD_{low} n = 41		No-CLD_{low} n = 100	
	$P_{low(NO)}$ FEV_1/FVC ratio <0.8 (Non-obstructive)	$P_{low(O)}$ FEV_1/FVC ratio <0.8 (Obstructive)	$CLD_{low(NO)}$ FEV_1/FVC ratio <0.8 (Non-obstructive)	$CLD_{low(O)}$ FEV_1/FVC ratio <0.8 (Obstructive)	No-$CLD_{low(NO)}$ FEV_1/FVC ratio <0.8 (Non-obstructive)	No-$CLD_{low(O)}$ FEV_1/FVC ratio <0.8 (Obstructive)
Subjects (n)	83 (59%) [a]	58 (41%) [a]	18 (44%) [b]	23 (56%) [b]	65 (65%) [c]	35 (35%) [c]
Family History						
A/N Maternal Smoking	8/82 (10%)	6/56 (11%)	3/18 (17%)	4/23 (17%)	5/64 (8%)	2/33 (6%)
P/N Maternal smoking	8/83 (10%)	8/57 (14%)	1/18 (6%)	6/23 (26%)	7/65 (11%)	2/34 (6%)
F/H Asthma	48/83 (58%)	35/57 (61%)	10/18 (56%)	11/23 (48%)	38/65 (59%)	24/34 (71%)
F/H Hay fever	43/82 (52%)	29/56 (52%)	8/17 (47%)	10/22 (46%)	35/65 (54%)	19/34 (56%)
F/H Eczema	32/82 (39%)	27/56 (48%)	5/17 (29%)	11/22 (50%)	27/65 (42%)	16/34 (47%)
F/H Allergies	34/82 (42%)	18/56 (32%)	5/17 (29%)	6/22 (27%)	29/65 (45%)	12/34 (35%)
Infant/Child History						
Bronchiolitis	22/83 (27%)	17/58 (29%)	7/18 (39%)	7/23 (30%)	15/65 (23%)	10/35 (29%)
Viral-induced wheeze	8/83 (10%) §	14/58 (24%)	0/18 (0%)	5/23 (22%)	8/65 (12%)	9/35 (26%)
Pneumonia	6/83 (7%)	7/58 (12%)	2/18 (11%)	3/23 (13%)	4/65 (6%)	4/35 (11%)
Wheeze-ever	42/81 (52%) §§	45/57 (79%)	10/18 (56%)	18/23 (78%)	32/63 (51%) ¥¥	27/34 (79%)
Wheeze last 12 months	18/83 (22%) §§§	30/58 (52%)	2/18 (11%) ℓ	10/23 (44%)	16/65 (25%) ¥¥	20/35 (57%)
Inhalers last 12 months	9/83 (11%) §§§	23/58 (40%)	0/18 (0%)	5/23 (22%) Ш	9/65 (14%) ¥¥¥	18/35 (51%)
Diagnosed Asthma	13/83 (16%) §§	22/57 (39%)	2/18 (11%)	5/23 (22%)	11/65 (17%) ¥¥	17/34 (50%)

[a] % of preterm-born with low lung function, [b] % of preterm-born with CLD and low lung function, [c] % of preterm-born without CLD and low lung function. Results are expressed as mean (95% CI), unless specified.

$P_{low(NO)}$ v $P_{low(O)}$ **§p<0.05, §§p<0.01, §§§p<0.001** **$CLD_{low(NO)}$ v $CLD_{low(O)}$** **ℓp<0.05, ℓℓp<0.01, ℓℓℓp<0.001** **No-$CLD_{low(NO)}$ v No-$CLD_{low(O)}$** **¥p<0.05, ¥¥p<0.01, ¥¥¥p<0.001. $CLD_{low(NO)}$ v No-$CLD_{low(NO)}$** **ʒp<0.05, ʒʒp<0.01, ʒʒʒp<0.001**
$CLD_{low(O)}$ v No-$CLD_{low(O)}$ **Шp<0.05, ШШp<0.05, ШШШp<0.001**

Abbreviations: IUGR = intrauterine growth restriction, PROM = prolonged rupture of membranes, C/S = caesarean section delivery, CLD = Chronic lung disease, ROP = retinopathy of prematurity, NEC = necrotising enterocolitis requiring treatment, PDA = patent ductus arteriosus requiring medical or surgical treatment, Combined Illness = IVH or ROP or NEC in the neonatal period, Chest drain = insertion to treat pneumothorax, A/N = antenatal and P/N = postnatal, FH = family history.

3.5.2 Lung function and respiratory phenotypes

Of the 141 children with a $\%FEV_1$ ≤85%, 134 completed both pre- and post-spirometry measures. 6 children (7%) in the $P_{low(O)}$ group and 1 child (2%) in the $P_{low(NO)}$ group did not complete post-bronchodilator spirometry. Attrition between CLD groups were comparable irrespective of respiratory phenotype.

17 children with baseline spirometry were unable to complete FeNO testing: 9 (16%) children in the $P_{low(O)}$ group and 8 (10%) children in the $P_{low(NO)}$ group. In those with CLD, 6 (26%) of the $CLD_{low(O)}$ group and 1 (6%) of those in the $CLD_{low(NO)}$ group did not complete FeNO testing. This compares with 3 (9%) of those in the No-$CLD_{low(O)}$ group and 7 (11%) of those in the No-$CLD_{low(NO)}$ group.

Details of lung function testing are shown in Figure 3-6 and Table 3-17. Compared to the $P_{low(NO)}$ group, the $P_{low(O)}$ group had lower baseline $\%FEV_1$ (79.1 vs 70.5, p<0.001) and $\%FEF_{25-75\%}$ (42.9 vs 66.8, p<0.001). Conversely, the $P_{low(NO)}$ group had a lower %FVC (80.7 vs 87.6, p<0.001).

The observation that children with an obstructive pattern of lung function deficits had lower baseline spirometry measures except %FVC compared to those with non-obstructive pattern was consistent across all groups irrespective of CLD status. In both obstructive and non-obstructive groups, children with CLD had lower spirometry measures compared to those without CLD. These findings were, however, not statistically different.

3.5.3 BDR and FeNO

Post-bronchodilator increases were observed in all spirometry measures in both the obstructive and non-obstructive groups. The $P_{low(O)}$ group had the largest mean increase in $\%FEV_1$ from 70.5% to 82.9% (mean change 12.4%, 95%CI 9.9 to 14.8%) resulting in comparable post-bronchodilator $\%FEV_1$ measures between the $P_{low(O)}$ and $P_{low(NO)}$ group (82.9% vs 83.7%). Despite the $P_{low(O)}$ group also having the largest increase in mean $\%FEF_{25-75\%}$ from 42.9% to 61.0% (mean change 18.2%, 95%CI 15.5 to 20.9%), the post-bronchodilator $\%FEF_{25-75\%}$ remained significantly lower than the $P_{low(NO)}$ group (61% vs 79.8%, p<0.001). The higher mean %FVC change from 87.6% to 92.7% (mean change 4.8%, 95%CI 2.4 to 7.3%) observed in the $P_{low(O)}$ group compared with a change from 80.7% to 82.8% (mean change 1.6%, 95%CI 0.56 to 2.7%) in the $P_{low(NO)}$ group resulted in the difference in FEV_1/FVC ratio between groups remaining highly significant (0.79 vs 0.89,

$p<0.001$). The increase in $\%FEV_1$, $\%FEF_{25-75\%}$ and %FVC observed in the $P_{low(O)}$ group after inhalation of bronchodilator is consistent with reversible airways disease.

The higher proportion of those with a positive BDR in the $P_{low(NO)}$ group compared with those in the $P_{low(NO)}$ group (53% vs 12%, $p<0.001$) was consistent with the higher mean change of $\%FEV_1$ observed in the $P_{low(O)}$ group. This increased proportion of children with a positive BDR was observed in the two obstructive groups with and without CLD.

A higher proportion of those in the $P_{low(O)}$ group also had a FeNO >35ppb when comparing to the $P_{low(NO)}$ group (33% vs 16%, $p<0.05$). Interestingly, when prior CLD status was considered, the proportions of those in the obstructive group with a FeNO >35ppb changed. Whilst not statistically significant, the No-$CLD_{low(O)}$ group had a higher proportion of children with a FeNO >35ppb compared to those with the $CLD_{low(O)}$ group (41% vs 18%) (Table 3-17).

Figure 3-6 Respiratory phenotypes, CLD, BDR and FeNO levels

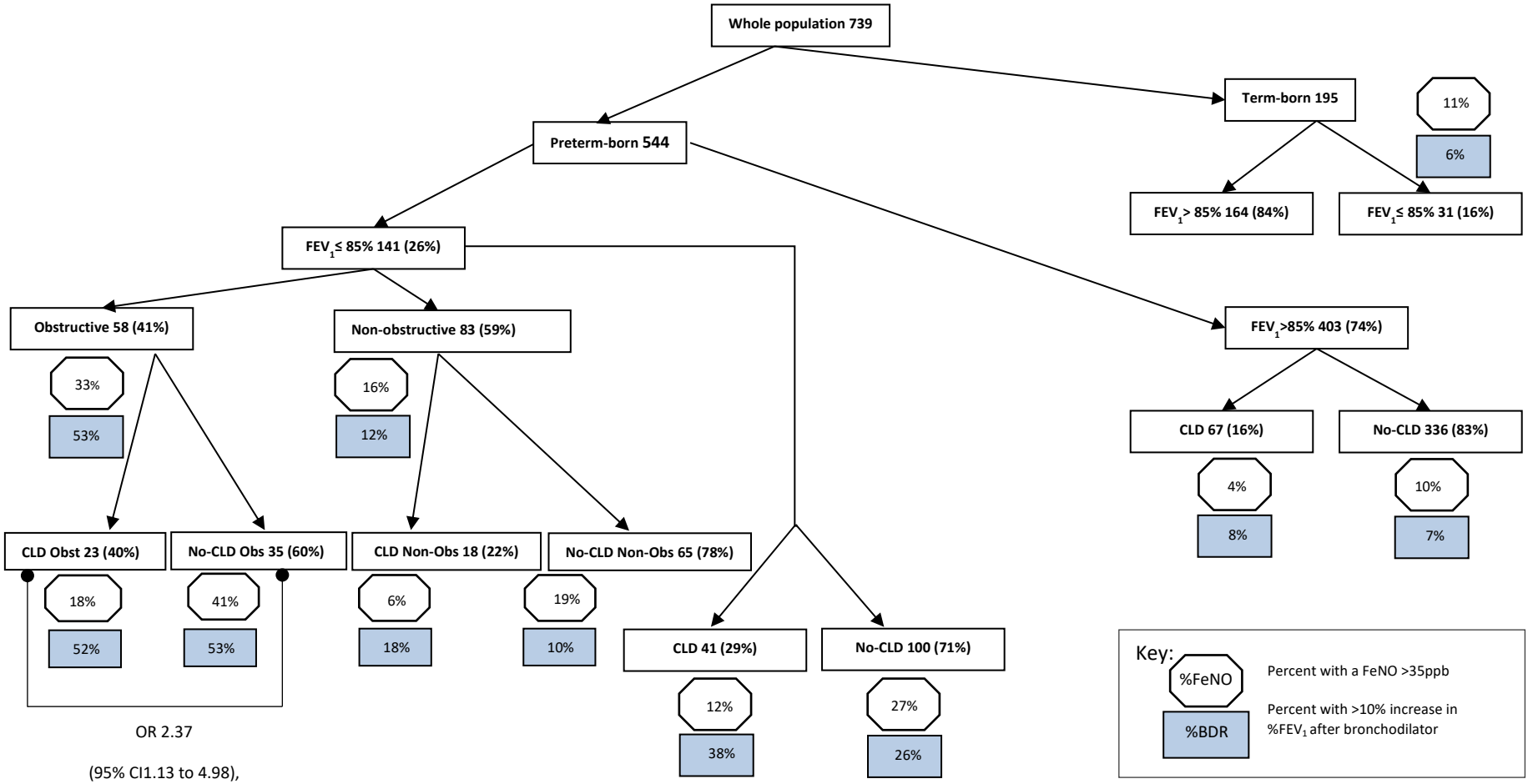


Table 3-17 Lung function by respiratory phenotype

	P_{low} n = 141 Mean age 9.9 years		Preterm-born population with % predicted FEV_1 ≤85% (P_{low}) n = 141. Mean age 9.9 years			
			CLD_{low} n = 41 Mean age 10.2 years		**No-CLD_{low} n = 100 Mean age 9.8 years**	
	$P_{low(NO)}$ (Non-obstructive)	**$P_{low(O)}$ (Obstructive)**	**$CLD_{low(NO)}$ (Non-obstructive)**	**$CLD_{low(O)}$ (Obstructive)**	**No-$CLD_{low(NO)}$ (Non-obstructive)**	**No-$CLD_{low(O)}$ (Obstructive)**
Baseline spirometry	**n = 83**	**n = 58**	**n = 18**	**n = 23**	**n = 65**	**n = 35**
% predicted FEV_1	79.1 (78.0 to 80.2) §§§	70.5 (67.5 to 73.5)	77.4 (74.3 to 80.5) ℓℓ	68.6 (63.5 to 73.8)	79.6 (78.4 to 80.8) ¥¥¥	71.8 (68.0 to 75.6)
% predicted FVC	80.7 (79.3 to 82.2) §§§	87.6 (84.2 to 89.3)	79.6 (75.5 to 83.6)	84.1 (80.3 to 87.9)	81.1 (79.5 to 82.6) ¥¥¥	88.5 (85.0 to 92.0)
FEV_1/FVC ratio	0.86 (0.85 to 0.87) §§§	0.71 (0.69 to 0.73)	0.85 (0.83 to 0.87) ℓℓℓ	0.71 (0.68 to 0.74)	0.86 (0.85 to 0.88) ¥¥¥	0.71 (0.68 to 0.73)
% predicted $FEF_{25-75\%}$	66.8 (64.4 to 69.3) §§§	42.9 (39.9 to 46.0)	64.2 (59.6 to 68.5) ℓℓℓ	41.1 (36.0 to 46.3)	67.6 (64.7 to 70.5) ¥¥¥	44.1 (40.1 to 48.1)
Post-BD spirometry	**n = 77**	**n = 57**	**n = 17**	**n = 23**	**n = 60**	**n = 34**
% predicted FEV_1	83.7 (81.9 to 85.0)	82.9 (79.9 to 85.8)	81.9 (76.3 to 87.6)	81.7 (77.7 to 85.8)	84.2 (82.5 to 85.9)	83.6 (79.3 to 87.9)
% predicted FVC	82.8 (80.8 to 84.3) §§§	92.7 (88.8 to 94.6)	81.9 (77.2 to 86.6) ℓℓ	90.9 (86.6 to 95.2)	82.7 (80.9 to 84.6) ¥¥¥	92.3 (88.2 to 96.4)
FEV_1/FVC ratio	0.89 (0.88 to 0.90) §§§	0.79 (0.77 to 0.81)	0.87 (0.84 to 0.91) ℓℓℓ	0.79 (0.76 to 0.82)	0.89 (0.88 to 0.90) ¥¥¥	0.79 (0.76 to 0.82)
% predicted $FEF_{25-75\%}$	79.8 (76.3 to 83.2) §§§	61.0 (57.2 to 64.7)	74.4 (65.7 to 83.0) ℓℓ	59.0 (54.3 to 63.7)	81.3 (77.6 to 85.1) ¥¥¥	62.3 (56.6 to 67.9)
Mean Change in % predicted spirometry ##	**n = 77**	**n = 57**	**n = 17**	**n = 23**	**n = 60**	**n = 34**
FEV_1	4.6 (3.3 to 6.0) §§§	12.4 (9.9 to 14.8)	4.5 (1.1 to 8.0) ℓℓ	13.1 (9.5 to 16.8)	4.7 (3.2 to 6.1) ¥¥¥	11.8 (8.5 to 15.2)
FV	1.6 (0.56 to 2.7) §	4.8 (2.4 to 7.3)	2.1 (-0.2 to 4.5)	6.8 (2.2 to 11.4)	1.5 (0.26 to 2.7)	3.5 (0.76 to 6.3)
FEV_1/FVC ratio	0.031 (0.021 to 0.041) §§§	0.084 (0.069 to 0.099)	0.023 (-0.006 to 0.052) ℓℓ	0.078 (0.055 to 0.101)	0.033 (0.022 to 0.044) ¥¥¥	0.088 (0.068 to 0.110)
$FEF_{25-75\%}$	13.5 (10.9 to 16.1)§	18.2 (15.5 to 20.9)	10.2 (4.2 to 16.3) ℓ	17.9 (14.4 to 21.3)	14.5 (11.6 to 17.3)	18.4 (14.3 to 22.5)
Positive BDR	9 (12%) §§§	30 (53%)	3 (18%) ℓ	12 (52%)	6 (10%) ¥¥¥	18 (53%)
FeNO	**n = 75**	**n = 49**	**n = 17**	**n = 17**	**n = 58**	**n = 32**
FeNO >35ppb	12 (16%) §	16 (33%)	1 (6%)	3 (18%)	11 (19%) ¥	13 (41%)
FeNO>30ppb	12 (16%) §§	18 (37%)	1 (6%)	4 (24%)	11 (19%) ¥	14 (44%)

95% confidence intervals or percentages shown in brackets. ## Mean change in spirometry readings based on all children with pre- and post-BD spirometry

$P_{low(NO)}$ v $P_{low(O)}$ §p<0.05, §§p<0.01, §§§p<0.001 $CLD_{low(NO)}$ v $CLD_{low(O)}$ ℓp<0.05, ℓℓp<0.01, ℓℓℓp<0.001 No-$CLD_{low(NO)}$ v No-$CLD_{low(O)}$ ¥p<0.05, ¥¥p<0.01, ¥¥¥p<0.001

$CLD_{low(NO)}$ v No-$CLD_{low(NO)}$ ʒp<0.05, ʒʒp<0.01, ʒʒʒp<0.001 $CLD_{low(O)}$ v No-$CLD_{low(O)}$ Ɯp<0.05, ƜƜp<0.05, ƜƜƜp<0.001

3.5.4 Early life factors associated with different respiratory phenotypes in preterm-born children

The use of the FEV_1/FVC ratio has helped me describe two respiratory phenotypes in children with a $\%FEV_1$ ≤85% . This is a strategy which can easily be used in routine clinical practice where hand-held spirometry is readily accessible - including in GP practices. Having previously demonstrated that CLD is a poor predictor of lung function deficits in childhood, it is of interest that – in those with a $\%FEV_1$ ≤85% – children with CLD are 2.37 times more likely to have lung function deficits that are obstructive in nature than those without CLD. Modelling to further understand the impact of CLD on the development of obstructive lung function deficits and identify significant early life factors that may predispose preterm-born children to obstructive and non-obstructive respiratory phenotypes was undertaken.

Initial univariable logistic regression included significant demographic variables or early life factors felt to be clinically relevant. Using the binary outcome of FEV_1/FVC <0.8 or ≥0.8, modelling was performed separately for both non-obstructive and obstructive lung function deficit groups with preterm-born children with a $\%FEV_1$>85% used as the control group (PTC). These early life factors for both non-obstructive and obstructive phenotypes are detailed in Table 3-18 and Table 3-19 respectively. CLD (all cases), CLD_{36} and CLD_{28} were separately analysed to evaluate their association with both obstructive and non-obstructive respiratory phenotypes.

A significant inverse relationship with gestational age was observed in both obstructive (Beta -0.165, p<0.001) and non-obstructive (Beta -0.144, p<0.001) groups. Those with obstructive lung function deficits had significant associations with additional early life factors; IUGR (OR 2.58, 95%CI 1.33 to 4.99), PDA (OR 2.50, 95%CI 1.01 to 6.18), NEC (OR 3.89, 95%CI 1.65 to 9.11) and CLD (OR 3.30 95%CI 1.83 to 5.93). On further analysis CLD_{36} was a significant covariate (OR 3.27 95%CI 1.70 to 6.33), but CLD_{28} was not, suggesting those with more severe disease have greater risk for obstructive lung function deficits.

Table 3-18 Univariable analysis of predictors for non-obstructive lung function deficits

Participants n=83			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	**-0.144**	**0.044**	**0.001**
Factors	**Beta**	**Odds Ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	0.317	1.37 (0.71 to 2.67)	0.349
CLD (ref = No)	0.328	1.39 (0.77 to 2.49)	0.271
CLD_{36} (ref = No)	0.163	1.18 (0.57 to 2.45)	0.663
PDA (ref = No)	0.489	1.63 (0.67 to 3.98)	0.282
NEC (ref = No)	0.840	1.09 (0.36 to 3.30)	0.882
CLD_{28} (ref = No)	0.478	1.61 (0.70 to 3.71)	0.261
FH asthma* (ref = No)	0.180	1.20 (0.74 to 1.93)	0.460
Sex (ref = Female)	-0.312	0.73 (0.46 to 1.18)	0.198
AN maternal smoking* (ref = No)	-0.249	0.78 (0.35 to 1.72)	0.536
PN maternal smoking* (ref = No)	-0.467	0.63 (0.29 to 1.37)	0.241
PROM* (ref = No)	-0.267	0.77 (0.46 to 1.27)	0.304
LSCS* (ref = No)	0.200	1.22 (0.75 to 1.98)	0.418
Covariables presented as Beta value and SE. Beta value and Odds Ratios with (95%CI) presented for all factors.			

Table 3-19 Univariable analysis of predictors for obstructive lung function deficits

Participants n=58			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	**-0.165**	**0.049**	**0.001**
Factors	**Beta**	**Odds Ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	0.948	**2.58 (1.33 to 4.99)**	**0.005**
CLD (ref = No)	1.193	**3.30 (1.83 to 5.93)**	**<0.001**
CLD_{36} (ref = No)	1.186	**3.27 (1.70 to 6.33)**	**<0.001**
PDA (ref = No)	0.915	**2.50 (1.01 to 6.18)**	**0.048**
NEC (ref = No)	1.355	**3.89 (1.65 to 9.11)**	**0.002**
CLD_{28} (ref = No)	0.730	2.08 (0.85 to 5.04)	0.107
FH asthma* (ref = No)	0.329	1.39 (0.79 to 2.45)	0.257
Sex (ref = Female)	0.044	1.05 (0.60 to 1.81)	0.876
AN maternal smoking* (ref = No)	-0.145	0.87 (0.35 to 2.13)	0.752
PN maternal smoking* (ref = No)	-0.041	0.96 (0.43 to 2.13)	0.920
PROM* (ref = No)	0.267	1.31 (0.74 to 2.30)	0.354
LSCS* (ref = No)	-0.335	0.72 (0.41 to 1.24)	0.234
Covariables presented as Beta value and SE. Beta value and Odds Ratios with (95%CI) presented for all factors.			

Subsequent multivariable modelling was undertaken including early life factors with a significance level of <0.10 on univariable analysis using the enter method. Due to the lack of multiple significant covariables in the non-obstructive group, further multivariable analysis to identify associations with the development of lung function deficits was limited to the obstructive group. To ensure greater understanding of the impact of disease severity on the development of obstructive lung function deficits, CLD (all cases) and CLD_{36} were used in separate models.

Binary logistic regression was undertaken using significant early life factors identified during univariate analysis using CLD (all cases) and CLD_{36} (Table 3-20, Table 3-21).

Table 3-20 Multivariable modelling for predictors of obstructive lung function deficits with all cases of CLD *(ENTER method)*

Participants n=461 (CLD n = 90)			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	-0.089	0.078	0.252
Factors	**Beta**	**Odds Ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	0.688	1.99 (0.97 to 4.08)	0.061
CLD (ref = No)	0.776	2.05 (0.81 to 5.22)	0.132
NEC (ref = No)	0.717	2.17 (0.84 to 5.60)	0.109
PDA (ref = No)	-0.247	0.78 (0.26 to 2.32)	0.657
Covariables presented as Beta value and SE. Beta value and Odds Ratios with (95% CI) presented for all factors.			

Table 3-21 Multivariable modelling for predictors of obstructive lung function deficits in those with CLD_{36} *(ENTER method)*

Participants n=461 (CLD_{36} n = 58)			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	**-0.142**	**0.069**	**0.041**
Factors	**Beta**	**Odds Ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	0.682	1.98 (0.96 to 4.07)	0.064
CLD_{36} (ref = No)	0.309	1.36 (0.53 to 3.54)	0.525
PDA (ref = No)	-0.202	0.82 (0.26 to 2.53)	0.727
NEC (ref = No)	0.848	2.34 (0.91 to 6.02)	0.079
Covariables presented as Beta value and SE. Beta value and Odds Ratios with (95% CI) presented for all factors.			

Children with CLD_{36} and obstructive lung function deficits had a significant association with gestational age (Table 3-21). However, when analysing children in the obstructive group with CLD (all cases) there were no significant covariables identified (Table 3-20).

Early life factors with a significance level of <0.10 on univariable analysis were further modelled using the forward method. This showed that when including the variable CLD (all cases), the only significant early life factor associated with obstructive lung function deficits was CLD (Table 3-22). Conversely modelling limited to those with CLD_{36} showed a significant association between gestation at birth and IUGR and obstructive lung function deficits but did not show any significant association with CLD_{36} (Table 3-23).

Table 3-22 Multivariable modelling for predictors of obstructive lung function deficits using all cases of CLD *(FORWARD method)*

Participants n=461 (CLD n = 90)			
Factors	**Beta**	**Odds Ratio (95% confidence interval)**	**Significance**
CLD	**1.270**	**3.56 (1.96 to 6.47)**	**<0.001**
Beta value and Odds Ratios with (95% CI) presented for all factors.			

Table 3-23 Multivariable modelling for predictors of obstructive lung function deficits using CLD_{36} *(FORWARD method)*

Participants n=461 (CLD_{36} n = 58)			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	**-0.185**	**0.050**	**<0.001**
Factors	**Beta**	**Odds Ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	0.743	**2.10 (1.04 to 4.27)**	**0.039**
Covariables presented as Beta value and SE. Beta value and Odds Ratios with (95% CI) presented for all factors.			

Additional exploratory modelling comparing early life factors between obstructive with non-obstructive respiratory phenotypes using the enter method was also undertaken. Early life factors with a significance level of <0.10 on univariable analysis in the obstructive group were used in the modelling. CLD and CLD_{36} were analysed separately to identify whether disease severity has any association with the development of the obstructive respiratory phenotype. Details of the modelling are in Table 3-24 and Table 3-25.

Table 3-24 Multivariable analysis of early life factors, including all CLD, associated with obstructive lung function deficits compared with non-obstructive lung function deficits *(ENTER method)*

Participants n= 141 (CLD n= 41)			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	0.153	0.095	0.105
Factors	**Beta**	**Odds ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	0.608	1.84 (0.73 to 4.73)	0.208
CLD (ref = No)	**1.650**	**5.21 (1.50 to 18.04)**	**0.009**
NEC (ref = No)	0.999	2.72 (0.72 to 10.24)	0.140
PDA (ref = No)	-0.290	0.75 (0.21 to 2.70)	0.660
Covariables presented as Beta value and SE. Beta value and Odds Ratios with (95% CI) presented for all factors.			

Table 3-25 Multivariable analysis of early life factors, including CLD_{36}, associated with obstructive lung function deficits compared with non-obstructive lung function deficits *(ENTER method)*

Participants n= 141 (CLD_{36} n= 26)			
Covariables	**Beta**	**Standard error**	**Significance**
Gestation	0.062	0.077	0.422
Factors	**Beta**	**Odds ratio (95% confidence interval)**	**Significance**
IUGR (ref = No)	0.369	1.45 (0.58 to 3.64)	0.431
CLD_{36} (ref = No)	**1.295**	**3.65 (1.14 to 11.74)**	**0.030**
NEC (ref = No)	1.025	2.79 (0.76 to 10.27)	0.140
PDA (ref = No)	-0.219	0.80 (0.21 to 3.05)	0.748
Covariables presented as Beta value and SE. Beta value and Odds Ratios with (95% CI) presented for all factors.			

Comparison of early life factors in those with obstructive lung function deficits to those with non-obstructive deficits showed a significant association between CLD and obstructive lung function deficits. Children with a $\%FEV_1$ ≤85% who had any historical diagnosis of CLD were over 5 times more likely to develop obstructive lung function deficits (OR 5.21, 95%CI 1.50 to 18.04, p 0.009). Those with the more severe disease (CLD_{36}) were significantly more likely to develop obstructive lung function deficits compared with those who develop non-obstructive lung function deficits (OR 3.65, 95%CI 1.14 to 11.74, p 0.03).

3.5.5 Summary of results

I have used the FEV_1/FVC ratio to delineate preterm-born children with a $\%FEV_1$ ≤85% into two respiratory phenotypes - obstructive and non-obstructive. I have described how preterm-born children with evidence of obstructive lung function deficits have the lowest spirometry measures and the highest proportions of those with a positive BDR. I have also described how a higher proportion of the obstructive group had a FeNO >35ppb compared to the non-obstructive group. Furthermore, 41% of those in the obstructive group who did not have CLD had a FeNO >35ppb compared with only 18% of those in the obstructive group with CLD.

I have demonstrated that obstructive lung function deficits in this preterm-born population are associated with a greater response to bronchodilator compared to those with non-obstructive lung function deficits and that there are different early life factors associated with each respiratory phenotype. One of those early life factors is CLD. Whist in isolation CLD is not a good prognostic factor for childhood lung function deficits, in those with reduced $\%FEV_1$, it is associated with obstructive lung function deficits. Unlike those without CLD who have evidence of ongoing Th2 driven eosinophilic inflammation which may be attributed to "classical" asthma, those with CLD did not.

3.6 Spirometry and FeNO testing to identify respiratory phenotypes in preterm-born children

The use of $\%FEV_1$ in assessing airflow limitation and reversibility is well established. The negative correlation between baseline $\%FEV_1$ and change in $\%FEV_1$ post-bronchodilator in preterm-born children has previously been described (Broström et al. 2010). To my knowledge, the correlation between other baseline measures and airflow limitation reversibility have not been explored in the preterm-born population.

In addition to continuous spirometry measurements, I also used pragmatically chosen cut-off points - which are readily accessible in the community setting - to further assess the impact that preterm birth has on childhood lung function, to identify different respiratory phenotypes and describe their response to inhaled bronchodilator.

The use of cut-off values for diagnosing respiratory disease is well established in adult populations with asthma and COPD. More recently, NICE guidelines have supported the use of the same FEV_1/FVC value for use in diagnosing paediatric asthma (NICE 2017a). However, the use of FEV_1/FVC of 0.7 in the paediatric population has no evidence base and has been heavily criticised (Murray et al. 2017). Diagnosis of asthma incorporates the use of spirometry testing in conjunction with comprehensive history taking and respiratory symptoms assessment and as such, arbitrary cut-off values may be of little consequence. However, it may help to identify persistent lung function deficits in a preterm-born population who do not always recognise or report respiratory symptoms which can negatively impact on lifelong respiratory health.

There is a need to evaluate the relationship between baseline spirometry and response to bronchodilator, to identify optimum spirometry cut-off values in the preterm-born population which can inform community screening programmes to identify those with ongoing lung function deficits. Further use of spirometry to classify children into different respiratory phenotypes may help identify those who will benefit from inhaled treatments and enable targeted treatments.

I have used baseline spirometry measures of $\%FEV_1 \leq 85\%$ to classify those with significant lung function deficits. Further combined use of baseline $\%FEV_1 \leq 85\%$ with FEV_1/FVC ratio <0.8 to define obstructive and ≥ 0.8 non-obstructive has enabled me to describe two respiratory phenotypes in the preterm-born population. A greater proportion of the obstructive respiratory phenotype compared with the non-obstructive respiratory

phenotype had a positive BDR (53% vs 12%, p <0.001) and FeNO >35ppb (33% vs 16%, p<0.05). Thus, suggesting spirometry may be a useful tool to identify different respiratory phenotypes who have different underlying lung pathologies and therefore, have different responses to inhaled medications.

FeNO measurement has become an important complementary part of objective lung function assessment in adults and children with respiratory symptoms. The use of FeNO has been proven useful in the diagnosis of asthma in the paediatric population (Murray et al. 2017) and, alongside spirometry, is a central component of asthma diagnosis in children (NICE 2017a).

Whilst acknowledging that preterm-born children have higher incidence of lung function deficits than their term-born counterparts, there is no evidence to suggest these are related to familial atopy associated with asthma (Edwards et al. 2016). Thus, the increased incidence of lung function deficits cannot simply be attributed to asthma.

The association between $\%FEV_1$ and FeNO has previously been described in asthmatic children (Fielding et al. 2019). Whilst there are studies which suggest that FeNO levels in the preterm-born population are either lower or similar to their term-born counterparts (Baraldi et al. 2005; Course et al. 2019), the association between spirometry measures and FeNO levels, and the diagnostic value of FeNO in identifying those who will respond to inhaled bronchodilator is unclear.

In addition to using scatter plots to identify associations between different spirometry measures and the response to bronchodilator, correlation analysis was undertaken to describe the relationships between baseline spirometry measures, and both change in $\%FEV_1$ and FeNO in preterm-born participants. Assessment of effect size (r) was assessed as: small 0.10, medium 0.30 and large 0.50. Area Under the Receiver Operator Characteristic curves (AUROC) were used to evaluate the diagnostic value of spirometry measures in identifying those who have a positive BDR. This process was also used to assess the diagnostic value of FeNO in identifying those who are most likely to have a positive BDR. Using the binary outcome of a positive BDR (defined as an absolute increase of $\%FEV_1$ ≥10%) as the gold standard outcome measure for airflow reversibility, analyses of the discriminatory value of baseline spirometry measures and FeNO were undertaken.

Definitions of the diagnostic value of baseline spirometry and FeNO based on the AUC are detailed in Table 3-26. Additional use of Youden's J statistic enabled identification of the

optimal cut-off point for each measure in terms of optimal balance of sensitivity and specificity.

Table 3-26 Definitions of the diagnostic value of a test based on AUC

AUC	Diagnostic value
0.5-0.6	Fail
0.6 - 0.69	Poor
0.7 - 0.79	Fair
0.8 - 0.89	Good
0.9 - 1.0	Very good

3.6.1 BDR in those with obstructive and non-obstructive respiratory phenotypes

Figure 3-7 details the change in $\%FEV_1$ following 400mcg inhaled salbutamol in preterm-born children with non-obstructive and obstructive respiratory phenotypes. Using a cut-off point of >10% change in $\%FEV_1$ as the indicator of a positive BDR, a higher proportion of children in the obstructive group had evidence of reversible air flow limitation, with a greater proportion of the non-obstructive phenotype observed to have fixed air flow limitation.

Whilst it would be easy to conclude that the combination of $\%FEV_1$ and FEV_1/FVC is the optimal strategy to identify those who are most likely to respond to inhaled bronchodilator, the scatter plot identifies four groups of children; those with obstructive lung function deficits who have airflow limitation that is reversible or fixed, and those with non-obstructive lung function deficits who have airflow limitation that is reversible or fixed. Further analysis of baseline spirometry is required to understand if using a combination of $\%FEV_1 \leq 85\%$ and $FEV_1/FVC < 0.8$ is the optimal strategy to identify those most likely to have a positive BDR.

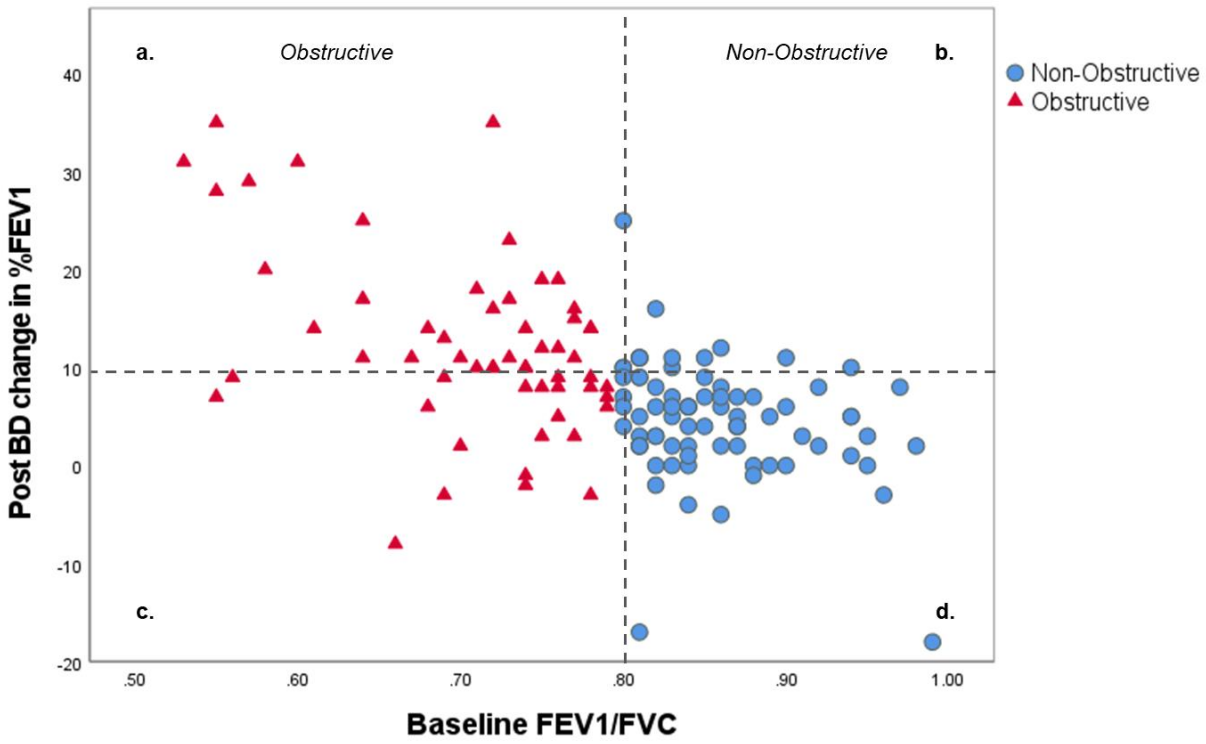


Figure 3-7 BDR in those with obstructive and non-obstructive respiratory phenotypes.

Vertical dotted line delineates obstructive and non-obstructive phenotypes. Horizontal dotted line at 10% mean %FEV_1 change delineates those with and without a positive BDR. Four groups of children identified a. obstructive with reversible airways disease, b. non-obstructive with reversible airways disease, c. obstructive with fixed airflow limitation, d. non-obstructive with fixed airflow limitation.

3.6.2 Spirometry measures and BDR in the whole preterm-born population

Highly significant negative correlations between baseline $\%FEV_1$ ($p<0.001$), $\%FEF_{25-75\%}$ ($p<0.001$) and FEV_1/FVC ($p<0.001$) with post-bronchodilator change in $\%FEV_1$ were found in the whole preterm-born group (Table 3-27). Baseline FEV_1 and $\%FEF_{25-75\%}$ had medium effect size (Pearson (r) -0.338 (95%CI -0.446 to -0.213) and -0.460 (95%CI -0.539 to -0.373) respectively). Baseline FEV_1/FVC had a large effect size (Pearson (r) -0.521, 95%CI -0.609 to -0.416) and the highest R^2 value of all measures. In contrast, there was no linear relationship between baseline %FVC and change in $\%FEV_1$, with little effect size (Pearson (r) 0.014, 95%CI -0.119 to 0.092).

Table 3-27 Correlation between baseline spirometry measures and change in $\%FEV_1$ in preterm-born children after bronchodilator.

Baseline spirometry measure	Pearson's Correlation Coefficient (r)	R^2
$\%FEV_1$	-0.338 (-0.446 to -0.213) ***	0.114
%FVC	-0.014 (-0.119 to 0.092)	N/A
FEV_1/FVC	-0.521 (-0.609 to -0.416) ***	0.271
$\%FEF_{25-75\%}$	-0.460 (-0.539 to -0.373) ***	0.211
Significance *p<0.05, **p<0.01, ***p<0.001, (Bias-corrected and accelerated (BCa) bootstrap 95%CI)		

Analysis of the diagnostic ability of baseline spirometry measures to identify a positive BDR in the whole preterm-born population are detailed in Figure 3-8 and Table 3-28. Mirroring the findings from the correlation analysis, baseline $\%FEV_1$ was a fair test (AUC of 0.758; 95%CI 0.694 to 0.822). Using a cut-off level of 85.5%, the baseline $\%FEV_1$ had a 79% sensitivity and 59% specificity. Baseline FEV_1/FVC and $\%FEF_{25-75\%}$ were both good tests (AUC 0.816, 95%CI 0.755 to 0.877 and AUC 0.825, 95%CI 0.772 to 0.878 respectively). The baseline FEV_1/FVC cut-off level of 0.815 had high sensitivity (76%) and specificity (77%). A cut-off point for baseline $\%FEF_{25-75\%}$ of 68.5% also resulted in high sensitivity and (71%) and specificity (80%) for positive BDR. As expected, baseline %FVC was a poor test (AUC 0.576, 95%CI 0.498 to 0.654).

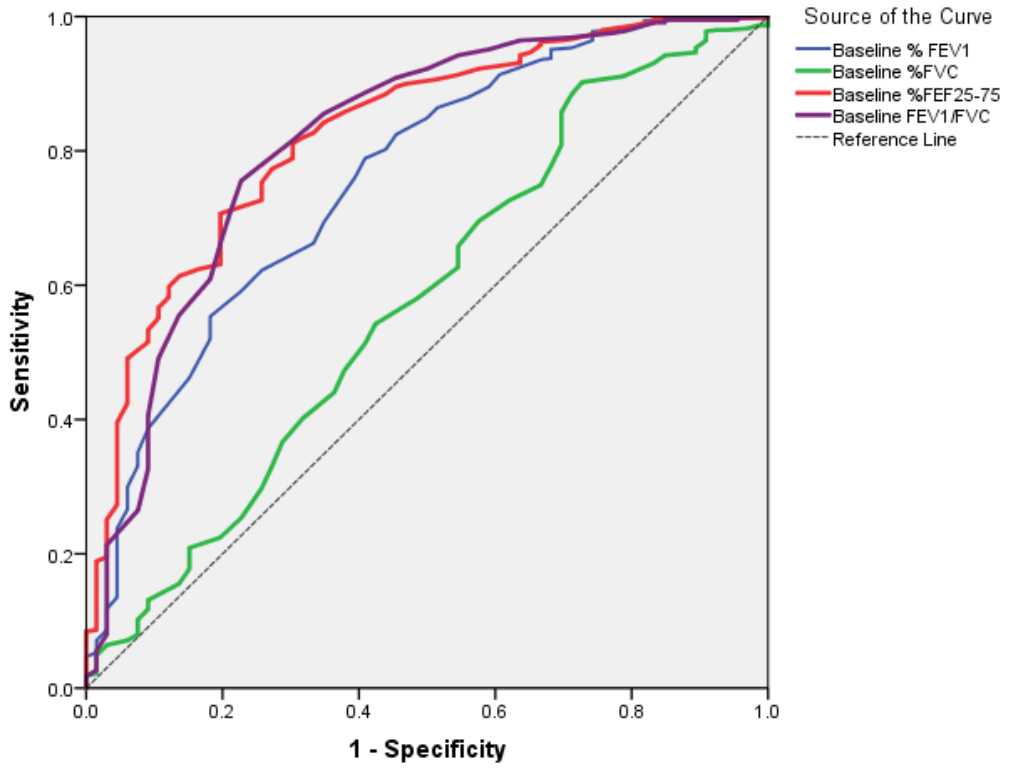


Figure 3-8 ROC Curves of spirometry measures to identify BDR in all preterm-born children.

Table 3-28 Sensitivity, specificity, and optimum cut-off levels for baseline spirometry measures and positive BDR

Baseline spirometry measure	Area under the curve (95% CI)	Sensitivity	Specificity	Youden's J Statistic for optimum cut-off level
%FEV_1	0.758 (0.694 to 0.822)	0.789	0.591	85.5%
%FVC	0.576 (0.498 to 0.654)	0.902	0.273	81.5%
FEV_1/FVC	0.816 (0.755 to 0.877)	0.756	0.773	0.815
$FEF_{25-75\%}$	0.825 (0.772 to 0.878)	0.707	0.803	68.5%

3.6.3 Spirometry measures and BDR in preterm-born children with a low %FEV_1

Further analysis of baseline spirometry measures in those with a %FEV_1 ≤85% was undertaken. Thus, evaluating the benefit of combining spirometry measures to identify those who have reversible airflow limitation and those who may benefit from inhaled bronchodilator.

The correlation between baseline spirometry measures and change in %FEV_1 in those with a %FEV_1 ≤85% were consistent with results observed in the whole preterm-born population (Table 3-29). There continued to be a highly significant negative correlation between baseline %FEV_1 and change in %FEV_1 following bronchodilator ($p<0.001$) with a medium effect size (Spearman's (r) -0.331, 95%CI -0.482 to -0.158). Both baseline FEV_1/FVC and %$FEF_{25-75\%}$ also had significant negative correlations ($p<0.001$) but with a large effect size (Spearman's (r) -0.546, 95%CI -0.669 to -0.398 and -0.530, 95%CI -0.654 to -0.382 respectively). As expected, poor correlation between baseline %FVC and post-bronchodilator change in %FEV_1 was observed with a non-significant p value and Spearman's (r) 0.156 (95%CI -0.04 to 0.295).

Table 3-29 Correlation between baseline spirometry measures and post-bronchodilator change in %FEV_1 in preterm-born children with a %FEV_1 ≤85% .

Baseline spirometry measure	Spearman's Correlation Coefficient (r)
%FEV_1	-0.331 (-0.486 to -0.153) **
%FVC	0.156 (-0.04 to 0.295)
FEV_1/FVC	-0.546 (-0.679 to -0.400) ***
%$FEF_{25-75\%}$	-0.530 (-0.649 to -0.380) ***
Significance *p<0.5, **p<0.01, ***p<0.001, (Bias-corrected and accelerated (BCa) bootstrap 95%CI)	

The diagnostic value of baseline spirometry measures in preterm-born children with a %FEV_1 ≤85% showed that baseline %FEV_1 became less useful, with an AUC 0.683 (95%CI 0.578 to 0.786). Using a cut-off value of 70.5%, baseline %FEV_1 continued to have high sensitivity (85.3%) but much lower specificity (41.0%). With an AUC of 0.775 (95%CI 0.689 to 0.861), the mid-expiratory flow measurement, baseline %$FEF_{25-75\%}$, was found to be a fair test. Like the baseline %FEV_1, it also had a high level of sensitivity (85.5%) with lower specificity (56.4%). Overall, baseline FEV_1/FVC was the optimal test (AUC 0.805; 95%CI 0.725 to 0.885) with a high level of sensitivity (75%) and specificity (72%). As expected, baseline FVC continued to be an unhelpful test with an AUC 0.435 (95%CI 0.320 to 0.550) (Table 3-30).

Table 3-30 Sensitivity, specificity, and optimum cut-off levels for baseline spirometry measures and positive BDR in those with a $\%FEV_1$ ≤85%.

Baseline spirometry measure	Area under the curve (95% CI)	Sensitivity	Specificity	Youden's J Statistic for optimum cut-off level
$\%FEV_1$	0.683 (0.578 to 0.786)	0.853	0.410	70.5%
%FVC	0.435 (0.320 to 0.550)	0.274	0.513	86.5%
FEV_1/FVC	0.805 (0.725 to 0.885)	0.747	0.769	0.785
$\%FEF_{25-75\%}$	0.775 (0.689 to 0.861)	0.853	0.564	47.5%

3.6.4 Spirometry measures in obstructive and non-obstructive respiratory phenotypes

I have demonstrated that - compared with $\%FEV_1$, %FVC and $\%FEF_{25-75\%}$ - the FEV_1/FVC ratio is the optimal spirometry measure to identify those most likely to have a positive BDR in children with a $\%FEV_1$ ≤85%.

The optimal cut-off points identified in the ROC curve analysis for both $\%FEV_1$ (85.5%) and FEV_1/FVC (0.79) are like previously used pragmatically chosen cut-off levels to characterise obstructive and non-obstructive respiratory phenotypes. Thus, suggesting that the use of baseline $\%FEV_1$ ≤85% and an FEV_1/FVC of <0.8 and ≥0.8 to identify those with lung function deficits and delineate into obstructive and non-obstructive respiratory phenotypes may more accurately identify those who would benefit from inhaled bronchodilator.

To further test this suggestion, analysis to more accurately describe the relationship between baseline spirometry measures and BDR in both obstructive and non-obstructive respiratory phenotypes was performed. Correlation analysis of baseline spirometry measures and change in $\%FEV_1$ post-bronchodilator for children in each phenotype was undertaken (Figure 3-9, Table 3-31).

The obstructive group had significant negative correlations with medium effect size between baseline $\%FEV_1$ ($p<0.01$, Spearman's (r) -0.371, 95%CI -0.605 to -0.213), $\%FEV_1/FVC$ ($p<0.01$, Spearman's (r) -0.355, 95%CI -0.588 to -0.082), $\%FEF_{25-75\%}$ ($p<0.01$, Spearman's (r) -0.403, 95%CI -0.628 to -0.125) and post-bronchodilator change in $\%FEV_1$. In the non-obstructive group, only the baseline $\%FEV_1/FVC$ had a significant negative correlation with the change in $\%FEV_1$ post-bronchodilator with a small effect size ($p<0.05$, Spearman's (r) -0.281, 95%CI -0.485 to 0.041).

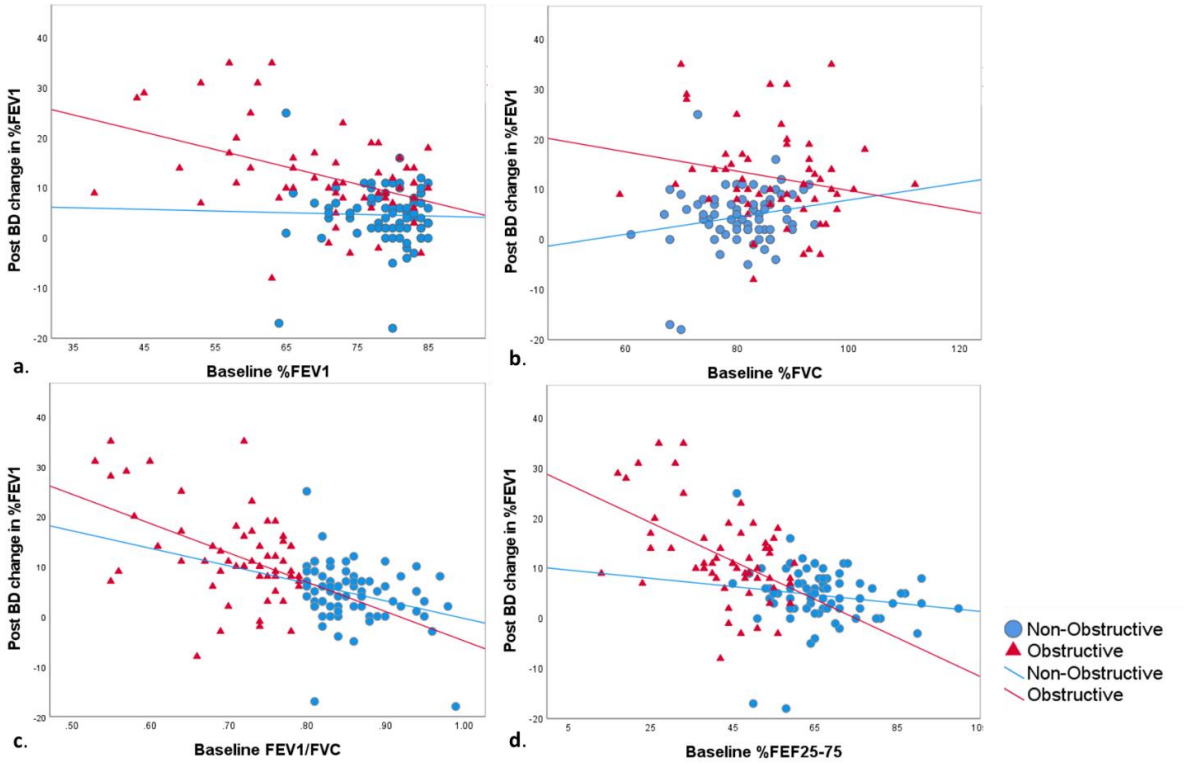


Figure 3-9 Baseline spirometry measures and correlation with mean change in $\%FEV_1$ in preterm-born children with non-obstructive and obstructive respiratory phenotypes.
a. Baseline $\%FEV_1$, b. Baseline FVC, c. Baseline $\%FEF_{25-75\%}$, c. Baseline FEV_1/FVC.

Table 3-31 Correlation between spirometry measures and respiratory phenotypes

Non-obstructive Group (n=77)		Obstructive Group (n=57)	
Baseline spirometry measure	**Spearman's Correlation Coefficient (r)**	**Baseline spirometry measure**	**Spearman's Correlation Coefficient (r)**
$\%FEV_1$	-0.069 (-2.86 to 0.158)	**$\%FEV_1$**	-0.371 (-0.605 to -0.213)**
%FVC	0.118 (-0.135 to 0.360)	**%FVC**	-0.204 (-0.449 to 0.061)
FEV_1/FVC	-0.281 (-0.485 to 0.041) *	**FEV_1/FVC**	-0.355 (-0.588 to -0.082)**
$\%FEF_{25-75\%}$	-0.223 (-0.431 to 0.006)	**$\%FEF_{25-75\%}$**	-0.403 (-0.628 to -0.125)**
Significance *p<0.05, **p<0.01, ***p<0.001, (Bias-corrected and accelerated (BCa) bootstrap 95%CI)			

Further AUROC analysis shows poor test diagnostic ability across all baseline spirometry measures once baseline $\%FEV_1$ and FEV_1/FVC have been used to delineate obstructive and non-obstructive phenotypes (Table 3-32 and Table 3-33).

Table 3-32 Sensitivity, specificity, and optimum cut-off levels for baseline spirometry measures to predict a positive BDR in the non-obstructive respiratory phenotype.

Baseline spirometry measure	Area under the curve (95% CI)	Sensitivity	Specificity	Youden's J Statistic for optimum cut-off level
$\%FEV_1$	0.530 (0.330 to 0.731)	0.412	0.778	81.5%
%FVC	0.446 (0.251 to 0.642)	0.529	0.556	81.5%
FEV_1/FVC	0.606 (0.432 to 0.781)	0.618	0.667	0.835
$\%FEF_{25-75\%}$	0.670 (0.498 to 0.842)	0.500	0.778	65.5%

Table 3-33 Sensitivity, specificity, and optimum cut-off levels for baseline spirometry measures to predict a positive BDR in the obstructive respiratory phenotype.

Baseline spirometry measure	Area under the curve (95% CI)	Sensitivity	Specificity	Youden's J Statistic for optimum cut-off level
$\%FEV_1$	0.643 (0.498 to 0.788)	0.778	0.533	70.0%
%FVC	0.577 (0.426 to 0.727)	0.852	0.400	81.5%
FEV_1/FVC	0.640 (0.495 to 0.785)	0.630	0.667	0.735
$\%FEF_{25-75\%}$	0.659 (0.517 to 0.800)	0.926	0.367	34.5%

Whilst the delineation of preterm-born children into obstructive and non-obstructive phenotypes identifies a clear difference in correlation between spirometry measures and response to bronchodilator, ROC curve analysis suggests that once placed into a respiratory phenotype, baseline spirometry measures lose their value as a diagnostic test for a positive BDR. Thus, there is no benefit in using additional baseline spirometry measures to identify those who will have a positive BDR.

3.6.5 Spirometry measures and FeNO in the preterm-born population

Having described the correlation between spirometry measures and post-bronchodilator change in $\%FEV_1$ in the preterm-born population, this section describes FeNO in the preterm-born population compared with term-born controls, explores the relationship between baseline spirometry measures and FeNO, and evaluates the role of FeNO as a diagnostic tool for identifying those who have a positive BDR.

3.6.5.1 FeNO measures in the preterm-born population

Table 3-34 outlines mean FeNO levels and proportions of children with a FeNO >35ppb between preterm-born children with different levels of lung function and term-born controls. No significant differences were found between the TC and PT groups when comparing mean FeNO (18.3ppb vs 17.3ppb respectively) or the proportion of children with a FeNO >35ppb (11% vs 13% respectively). Children in the P_{low} group had the highest mean FeNO level (21.8ppb) compared with the PTC (16.0 ppb) and TC (18.3ppb) groups. Whilst the difference in mean FeNO levels between the PTC and P_{low} groups was statistically (p,0.01) , the difference between the TC and P_{low} groups was not. The P_{low} group had a significantly higher proportion (23%) of children with a FeNO >35ppb, compared to the PTC group (9%, $p<0.001$), and TC group (11%, $p<0.01$).

Further analysis of FeNO measures between the obstructive and non-obstructive groups showed a notable, but non-significant difference in mean FeNO levels, with the obstructive group having a higher mean FeNO (25.8ppb) than the non-obstructive group (19.3ppb). A significant difference was also found when comparing the proportions of children in each group with a FeNO >35ppb, with 17% more of the obstructive group than the non-obstructive group having a FeNO >35ppb ($p<0.05$).

Table 3-34 FeNO levels in preterm- and term-born children

	Preterm-born population with % predicted FEV_1 ≤85% (P_{low}) n = 124		**Preterm-born population (≤34/40) (PT) n = 474**			
	$P_{low(NO)}$ FEV_1/FVC ratio <0.8 (Non-obstructive)	**$P_{low(O)}$ FEV_1/FVC ratio <0.8 (Obstructive)**	**≤34/40 % predicted FEV_1 ≤85 (P_{low})**	**≤34/40 control % predicted FEV_1 >85 (PTC)**	**≤34/40 Preterm-born (PT)**	**≥37/40 Term-born control (TC)**
FeNO	**n = 75**	**n = 49**	**n = 124**	**n = 350**	**n = 474**	**n = 183**
FeNO >35ppb	12 (16%) §	16 (33%)	28 (23%) ΘΘ,∞∞∞∞	33 (9%)	61 (13%)	20 (11%)
FeNO Mean	19.3 (14.2 to 24.4)	25.8 (19.8 to 31.9)	21.8 (17.9 to 25.7) ∞∞	16.0 (14.3 to 17.7)	17.5 (15.9 to 19.2)	18.3 (15.5 to 21.1)

95% confidence intervals or percentages shown in brackets. **Significance - PT vs TC √p<0.05, √√p<0.01, √√√p<0.001. P_{low} v PTC ∞p<0.05, ∞∞p<0.01, ∞∞∞∞p<0.001. P_{low} v TC Θp,0.05, ΘΘp<0.01, ΘΘΘp<0.001, ϷTC v TC ×p<0.05, ××p<0.01, ×××p<0.001. $P_{low(R)}$ v $P_{low(O)}$ §p<0.05, §§p<0.01, §§§p<0.001.**

3.6.5.2 The relationship between baseline spirometry and FeNO levels

There was no tangible relationship between baseline spirometry measures and FeNO in the whole preterm-born group. Despite this, baseline FEV_1/FVC was observed to have a significant negative correlation with FeNO ($p<0.05$). However, the effect size was small (Spearman's (r) -0.102, 95%CI -0.197 to -0.010). No significant correlations were found between all other baseline measures and FeNO level (Table 3-35).

Analysis of the preterm-born group with a $\%FEV_1 \leq 85\%$ showed no significant correlation between baseline $\%FEV_1$, %FVC, FEV_1/FVC or $\%FEF_{25-75\%}$ and FeNO level (Table 3-36). There were also no significant correlations between baseline spirometry measures and FeNO in both obstructive and non-obstructive respiratory phenotypes (Table 3-37).

Table 3-35 Correlation between baseline spirometry and FeNO in all preterm-born children

Baseline spirometry measure	Spearman's Correlation Coefficient (r)
$\%FEV_1$	-0.047 (-0.136 to 0.051)
%FVC	-0.022 (-0.114 to 0.069)
FEV_1/FVC	-0.102 (-0.197 to -0.010) *
$\%FEF_{25-75\%}$	-0.023 (-0.108 to 0.064)
Significance *p<0.05, **p<0.01, ***p<0.001, (Bias-corrected and accelerated (BCa) bootstrap 95%CI)	

Table 3-36 Correlation between baseline spirometry measures and change in $\%FEV_1$ in preterm-born children with air flow impairment.

Baseline spirometry measure	Spearman's Correlation Coefficient (r)
$\%FEV_1$	-0.018 (-0.199 to 0.158)
%FVC	0.035 (-0.142 to 0.223)
FEV_1/FVC	-0.173 (-0.356 to 0.022)
$\%FEF_{25-75\%}$	-0.063 (-0.258 to 0.133)
Significance *p<0.05, **p<0.01, ***p<0.001, (Bias-corrected and accelerated (BCa) bootstrap 95%CI)	

Table 3-37 Correlation between baseline spirometry measures and FeNO level in different respiratory phenotypes.

Non-obstructive Correlation (n=77)		Obstructive Correlation (n=57)	
Baseline spirometry measure	**Spearman's Correlation Coefficient (r)**	**Baseline spirometry measure**	**Spearman's Correlation Coefficient (r)**
$\%FEV_1$	0.064 (-0.004 to 0.105)	**$\%FEV_1$**	-0.050 (-0.336 to 0.233)
%FVC	0.063 (-0.116 to 0.236)	**%FVC**	-0.066 (-0.334 to 0.218)
FEV_1/FVC	-0.061 (-0.267 to 0.154)	**FEV_1/FVC**	-0.071 (-0.351 to 0.208)
$\%FEF_{25-75\%}$	0.123 (-0.093 to 0.317)	**$\%FEF_{25-75\%}$**	-0.050 (-0.331 to 0.239)
Significance *p<0.05, **p<0.01, ***p<0.001, (Bias-corrected and accelerated (BCa) bootstrap 95%CI)			

3.6.5.3 FeNO and BDR in the preterm-born population

Despite the absence of correlation between baseline spirometry and FeNO level, a scatterplot detailing the relationship between FeNO and change in $\%FEV_1$ post bronchodilator shows that a greater proportion of those with the obstructive phenotype had a positive BDR and a FeNO>35ppb. This suggests a greater proportion of those with the obstructive phenotype have reversible lung function deficits which may be due to Th2 driven eosinophilic inflammation compared to those with non-obstructive phenotype (Figure 3-10).

Further analysis demonstrated a highly significant positive correlation between post-bronchodilator change in $\%FEV_1$ and highest FeNO in those with obstructive lung function deficits with a medium effect size (Spearman's (r) 0.399, 95%CI 0.119 to 0.621). Conversely, there was no significant correlation observed in those with non-obstructive lung function deficits (Table 3-38).

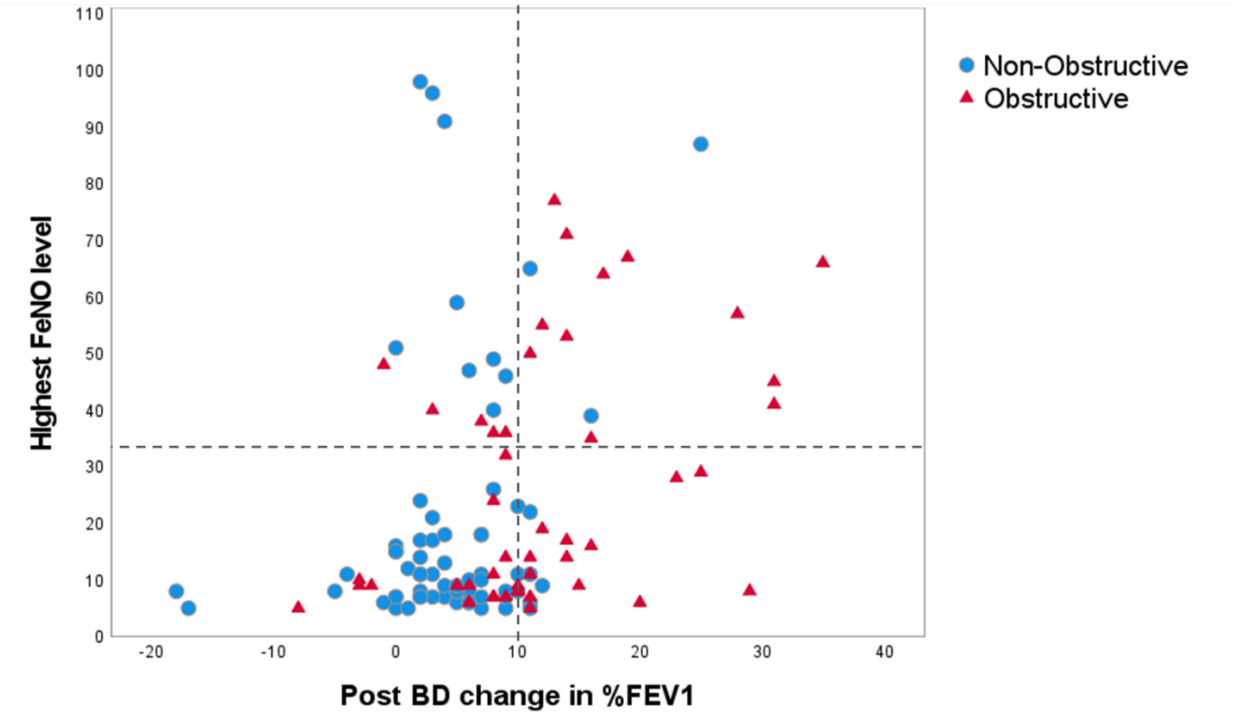


Figure 3-10 Relationship between FeNO levels and change in $\%FEV_1$ in the preterm-born population.
Positive BDR defined as change in $\%FEV_1$ of >10%. FeNO >35ppb (as per ATS guidelines).

Table 3-38 Correlation between change in %FEV_1 and FeNO >35ppb in two respiratory phenotypes

Non-obstructive Correlation (n=70)		Obstructive Correlation (n=48)	
	Spearman's Correlation Coefficient (r)		**Spearman's Correlation Coefficient (r)**
FeNO	0.139 (-0.130 to 0.396)	**FeNO**	0.399 (0.119 to 0.621)**
Significance *p<0.05, **p<0.01, ***p<0.001, (Bias-corrected and accelerated (BCa) bootstrap 95%CI)			

Mirroring the previous correlation analysis, both AUROC curve analysis demonstrate the poor diagnostic value of FeNO in identifying preterm-born children who will have a positive BDR in the whole preterm-born population enrolled in the study (AUC 0.659, 95%CI 0.571 to 0.746). Similar results were also observed in preterm-born children with a %FEV_1 ≤85% (AUC 0.693, 95%CI 0.578 to 0.808) and those with the non-obstructive respiratory phenotype (AUC 0.593, 95%CI 0.349 to 0.837). In those with the obstructive respiratory phenotype, FeNO measurement was a fair test to identify those who will have a positive BDR (AUC 0.729, 95%CI 0.584 to 0.874) with a high sensitivity (76%) but less useful specificity of 35% (Figure 3-11, Table 3-39). The optimal cut-off point in the obstructive respiratory phenotype was 12.5ppb.

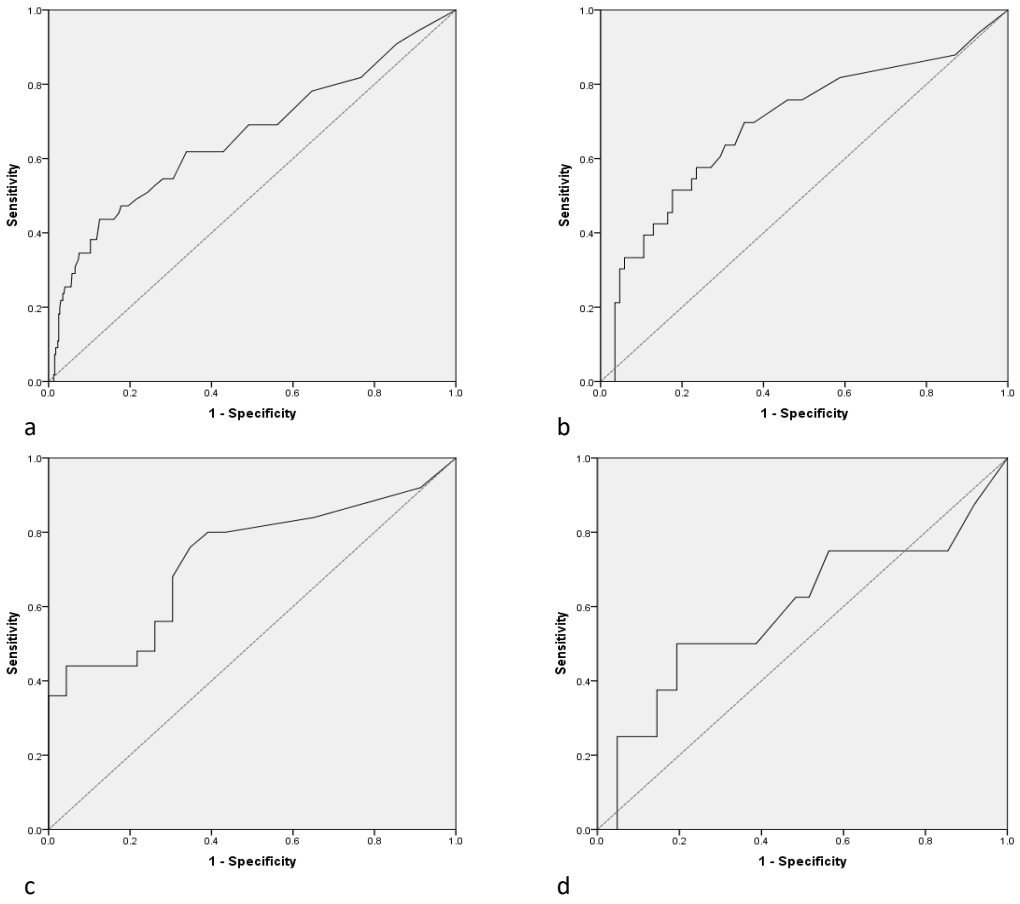


Figure 3-11 ROC curves of FeNO measurement to predict a positive BDR in preterm-born children.

a. Whole preterm-born population, b. Preterm with a %FEV_1 ≤85%*, c. Obstructive lung function deficits, d. Non-obstructive lung function deficits*

Table 3-39 AUC analysis of FeNO and BDR in preterm-born children

Population	Area under the curve (95% CI)	Sensitivity	Specificity	Youden's J Statistic for optimum cut-off level
Whole preterm-born population	0.659 (0.571 to 0.746)	0.436	0.125	27.5ppb
Preterm-born lung function deficits	0.693 (0.578 to 0.808)	0.274	0.134	25.5ppb
Obstructive respiratory phenotype	0.729 (0.584 to 0.874)	0.760	0.348	12.5ppb
Non-obstructive respiratory phenotype	0.593 (0.349 to 0.837)	0.500	0.194	21.5ppb

Summary of findings

Except for %FVC, all baseline spirometry measures obtained from preterm-born children in this large cohort had significant negative correlation with the change in $\%FEV_1$ following inhalation of a bronchodilator. Thus, there is an inverse relationship between baseline spirometry measures and increase in $\%FEV_1$ following bronchodilator in preterm-born children.

The combined use of baseline $\%FEV_1$ and FEV_1/FVC improved identification of those more likely to have a positive BDR, irrespective of the presence of respiratory symptoms. The combined use of these baseline spirometry measures has helped to identify at least two respiratory phenotypes who have a different response to bronchodilator in the preterm-born population. Using ROC analysis and Youden's J statistic, I have also described the ideal cut-off values for both FEV_1 and FEV_1/FVC in this population. These analyses support the earlier pragmatic values I chose to use.

With no difference in the FeNO levels between preterm- and term-born children, it is unlikely that the increased lung function deficits observed in the preterm-born population is entirely due to Th2 driven inflammation. However, there is some evidence that children with the obstructive respiratory phenotype have a greater risk for a FeNO >35ppb. Furthermore, there is some limited evidence that in children with a low $\%FEV_1$, FEV_1/FVC and a FeNO >35ppb are more likely to have a positive BDR, and therefore more likely to benefit from inhaled therapies.

4 Discussion

This chapter summarises the pertinent findings from my studies in the context of the existing literature. After reporting my findings, and discussing the strengths and limitations of my work, I will consider the implications of my findings for practice and future directions for research.

4.1 Findings

This section discusses my findings and addresses the hypotheses and specific aims outlined in chapter one of this thesis. Information is presented for of each hypothesis.

4.1.1 Preterm-born children have greater lung function deficits than term-born children.

I have accurately described lung function in a large population of children aged 7 – 12 years of age born at ≤34 weeks' gestation and compared them with term-born controls of equivalent age.

Despite the inclusion of moderately preterm-born children, at school-age these children continue to have lower spirometry measures and a greater response to a single dose of inhaled salbutamol than term-born controls, confirming the hypothesis that preterm-born children have greater lung function deficits compared to term-born children.

These findings are consistent with the existing research and suggest that - despite being born in an era of routine maternal antenatal corticosteroid administration, postnatal exogenous surfactant installation, judicious oxygen use and gentler ventilation techniques - prematurity continues to be associated with impaired lung function in childhood (Fawke et al. 2010; Kotecha et al. 2013; Verheggen et al. 2016; Simpson et al. 2017).

Whilst the preterm-born population had lower spirometry measures compared with term-born controls, measures for the whole preterm-born population were within normal limits. This suggests that there is a need to identify those in the preterm-born group who have clinically relevant lung function deficits. Using a $\%FEV_1$ of ≤85% as a proxy for the LLN, preterm-born children had a higher incidence of significant lung function deficits and a greater proportion had evidence of a positive BDR compared with term-born controls.

In addition to lower gestation at birth, several demographic details differed between the preterm- and term-born groups. As expected, those born preterm had lower birthweight and

poorer neonatal health. They also had greater incidence of CLD, IUGR, delivery by caesarean section, maternal antenatal and postnatal smoking, and experienced higher levels of deprivation.

IUGR and delivery by caesarean section have both been associated with reduced lung function in childhood (Kotecha et al. 2010; Kotecha et al. 2016; Ronkainen et al. 2016). Maternal antenatal smoking has been associated with the development of IUGR (Lawder et al. 2019), whilst postnatal exposure to environmental smoke is associated with early life respiratory illness and wheeze (Vanker et al. 2017). The elevated incidence of maternal antenatal smoking in the preterm-born group may have contributed to the higher incidence of IUGR, birth by caesarean section, and respiratory illness observed in the preterm-born group.

Whilst a higher proportion of preterm-born children were born prior to the introduction of the Welsh smoking ban compared with term-born controls, nearly 70% were born after this time. Data demonstrates that despite smoking in Wales decreasing since 2007, those in the most deprived WIMD quintile continue to have higher rates of smoking compared to those in the least deprived quintile (29.2% vs 12%)(PHW 2020). It is likely that the greater levels of deprivation observed in the preterm-born group had a greater influence on maternal smoking than the recruitment of children born prior to the introduction of the smoking ban to the study. Deprivation has also been associated with preterm delivery and poor long-term health outcomes (Taylor-Robinson et al. 2011; Wickham et al. 2016; Wilding et al. 2019).

The increased prevalence of respiratory illness in early childhood in the preterm-born group is also consistent with prior studies (Been et al. 2010; Edwards et al. 2015). The observed decrease in the proportion of preterm-born children reporting wheeze in the last 12 months compared with wheeze-ever suggests there may be improvement in respiratory symptoms with ascending age. This is consistent with prior studies who also describe resolution of early wheeze in preterm-born children (Kotecha et al. 2019 Edwards et al. 2016).

It is of concern that, despite a higher proportion of the preterm-born group experiencing respiratory symptoms in the last 12 months, only 60% of these children had received inhaled treatments. Although studies are contradictory, this finding has been previously described (Hennessy et al. 2008; Fawke et al. 2010). The lack of evidence into the efficacy of bronchodilator use in the preterm-born population has been widely acknowledged and may explain the observed proportion of preterm-born children in this study with current

respiratory symptoms who were not receiving any appropriate treatment (Kotecha et al. 2015; Duijts et al. 2020; Cristea et al. 2021)

In summary, spirometry results in my study have demonstrated that there continues to be a significant proportion of preterm-born children with persisting significant lung function deficits that are poorly understood and potentially under-treated.

There is a need to provide more robust information to help identify preterm-born children at risk for significant lung function deficits and to understand how to provide targeted treatments and advice for individual children.

4.1.2 Preterm-born children with a history of CLD in infancy have greater incidence of lung function deficits compared to term- and preterm-born children with no history of CLD.

Twenty percent of preterm-born children were noted to have CLD in infancy. Whilst this is within the reported international incidence of CLD of between 15% and 32% in children born 24^{+0} to 31^{+6} weeks' gestation, it is lower than the UK incidence of 32% (Shah et al. 2016). The development of CLD is inversely related to gestation at birth, the inclusion of children born beyond 31^{+6} weeks' gestation in my study most likely explains this finding.

The CLD group had the lowest spirometry measures compared to both the No-CLD and TC groups. Thus, confirming the hypothesis that preterm-born children with a history of CLD have greater incidence of a $\%FEV_1 \leq 85\%$ compared to term- and preterm-born children with no history of CLD. However, I also found that the No-CLD group had evidence of reduced spirometry compared with the TC group. Several similar studies have previously identified that, whilst preterm-born children with CLD have the lowest spirometry measures, many without CLD also have reduced spirometry measures when compared to term-born controls (Fawke et al. 2010; Kotecha et al. 2013; Simpson et al. 2017). Despite the findings in these studies, the authors did not gather any further details about these children, limiting understanding of which group of preterm-born children are at greatest risk for significant lung function deficits in childhood.

Using $\%FEV_1 \leq 85\%$ as a pragmatic cut off to identify the incidence of significant lung function deficits, I identified that the CLD group were three times more likely than term-born controls and twice as likely as preterm-born children without CLD to have a $\%FEV_1 \leq 85\%$. Preterm-born children without CLD were also one and a half times more likely to have a $\%FEV_1 \leq 85\%$

than term-born controls. With 62% of children with CLD having normal lung function it is reasonable to suggest that CLD may not be the optimal predictor of lung function deficits in the preterm-born population.

Twenty six percent of all preterm-born children - 38% with CLD and 23% children without CLD - and 16% of term-born controls had a $\%FEV_1 \leq 85\%$. This finding is consistent with Doyle et al who, despite using a lower $\%FEV_1$ (<75%) in a more limited preterm-born population (<28 weeks' gestation or <1000 grams), showed that 19.7% preterm-born children had lung function deficits. 27.3% of those had CLD and 15.2% did not (Doyle 2006). Similarly, in a population of school-aged children born at ≤32 weeks' gestation, Simpson et al described how 46.8% of those with CLD and 13.5% without CLD and had lung function deficits (Simpson et al. 2017).

My data also showed that the CLD group required longer periods of supplemental oxygen therapy, were born at the earliest gestational age and had the lowest birthweight. They also suffered from poorer health during the neonatal period and reported greater incidence of respiratory illness in infancy compared to both the No-CLD and TC groups. In addition to CLD diagnosis, several studies have also identified that duration of oxygen therapy, lower gestation, low birthweight, and poor neonatal health are associated with childhood lung function deficits in the preterm-born population (Halvorsen et al. 2006; Fawke et al. 2010; Simpson et al. 2017). It is reasonable to suggest that CLD may be one of many factors that influences childhood lung function.

The CLD group reported increased incidence of wheeze-ever and bronchiolitis in infancy. However, wheeze in the last 12 months, asthma diagnosis and inhaler use were similar between the No-CLD and CLD groups. Bröstrom et al noted that - despite children with CLD having a reduced $\%FEV_1$ - only 8 out of 19 reported symptoms (Broström et al. 2010). The lack of reported respiratory symptoms in preterm-born children with CLD with significant lung function deficits was also noted by Nordlund et al who suggest that – children with CLD may have a lack of perception due to lung compromise being ever-present as opposed to episodic symptoms associated with asthma (Nordlund et al. 2017). This lack of perception may explain the similar reported respiratory symptoms and treatment between the No-CLD and CLD group. Conversely, with evidence that lung development continues beyond the neonatal period (Narayanan et al. 2013) it is also reasonable to suggest that CLD may be a marker for respiratory health limited to the neonatal and infancy periods and that lack of symptoms may reflect improvements in respiratory health. Whether the lack of reported

respiratory symptoms is due to lack of perception or due to CLD being a poor prognostic factor for childhood lung function deficits, there is a need to consider ongoing lung function testing - such as community-based spirometry - to identify children with persistent lung function deficits.

Following inhalation of salbutamol, all groups had increases in spirometry measures. The CLD group had the greatest increases in $\%FEV_1$, %FVC and FEV_1/FVC compared to the PTC and TC groups. Despite this, all post-bronchodilator spirometry measures, except %FVC, remained significantly lower than the No-CLD and TC groups. In contrast, the No-CLD group had post-bronchodilator spirometry measures comparable with the TC group. Fawke et al describe similar findings in an extremely preterm-born cohort where those with CLD had the lowest baseline spirometry and largest increase in $\%FEV_1$ compared with those without CLD and term-born controls. They also describe how the post-bronchodilator $\%FEV_1$ in all preterm-born children remained below term-born controls but did not differentiate between CLD and No-CLD (Fawke et al. 2010).

Using an absolute increase in $\%FEV_1$ of >10% to indicate reversibility of airflow limitation, it was clear that a higher proportion of preterm-born children in both CLD and No-CLD groups showed evidence of reversibility when compared to term-born controls. However, despite 38% of the CLD and 23% of the No-CLD groups having a $\%FEV_1$ ≤85%, a lower proportion of both groups (19% and 11% respectively) had a positive BDR, suggesting a proportion of children have a degree of irreversible or fixed airflow limitation.

With 108 children with CLD, there was an opportunity for additional analysis of the impact of the severity of CLD. Children with CLD_{36} had the lowest spirometry measures and largest response to bronchodilator, although these differences were not statistically significant.

Both the CLD_{28} and CLD_{36} groups had lower baseline spirometry measures compared to the No-CLD and TC groups. Whilst the CLD_{36} group had the lowest baseline measures of all groups, measures were not significantly lower than those in the CLD_{28} group. Following 400mcg salbutamol, the CLD_{28} group had comparable spirometry measures with the No-CLD group. The CLD_{36} group continued to have reduced $\%FEV_1$, FEV_1/FVC and $\%FEF_{25-75\%}$ compared with the No-CLD and TC groups. However, there were no statistically significant differences in the proportion of children in each group with a positive BDR.

The proportion of children with a $\%FEV_1$ ≤85% in both the CLD_{28} and CLD_{36} groups was comparable, with 38% of children having a $\%FEV_1$ ≤85%. It may be reasonable to suggest that

- whilst both CLD sub-groups have the same proportion of children with a $\%FEV_1$ ≤85% - those with a $\%FEV_1$ ≤85% in the CLD_{36} group had larger lung function deficits than those with a $\%FEV_1$ ≤85% in the CLD_{28} group. There were additional early life factors that differed between the groups: the CLD_{36} group had greater incidence of IUGR, neonatal respiratory compromise, higher use of postnatal corticosteroids and home oxygen use – suggestive of longer duration of oxygen therapy.

Whilst my findings did not show that any significant differences in lung function based on severity of CLD, prior studies have reported the negative impact that severity of CLD has on spirometry measures. Um-Bergström et al reported a relationship between reduced spirometry measures ($\%FEV_1$, FEV_1/FVC, $\%FEF_{25-75\%}$) and severity of CLD (Um-Bergström et al. 2017). Halvorsen et al also described associated reductions in $\%FEV_1$ with increasing severity of CLD (Halvorsen et al. 2006). However, both studies have limitations: Um-Bergström et al had limited numbers of recruits and lacked clarity about which recruits had received surfactant. Similarly, whilst Halvorsen et al's study included two groups - one born in the pre- and one in the post-surfactant era - only 49% of those in the post-surfactant era had received surfactant as rescue therapy. Additionally, maternal antenatal corticosteroids were only administered in 44% of cases in the latter group. The benefits of administration of maternal antenatal corticosteroids and postnatal installation of surfactant for RDS have been described (Roberts et al. 2017; Sweet et al. 2019) and have resulted in their routine administration in preterm-born infants. Children enrolled into my study were born in an era when surfactant and antenatal maternal steroid administration were routine care and therefore the different populations studied may have different underlying lung pathologies.

In summary, whilst children with CLD had the lowest baseline spirometry measures and greatest incidence of a $\%FEV_1$ ≤85%, children without CLD also had evidence of significant ongoing lung function deficits compared to term-born controls. Disease severity did not impact the proportion of children with a $\%FEV_1$ ≤85%.

There is a need to examine the preterm-born population with evidence of lung function deficits irrespective of CLD status to improve understanding of early life factors that may negatively impact on childhood lung function.

4.1.3 CLD is the optimal predictor of lung function deficits in preterm-born school-aged children.

Analysis of early life factors associated with a $\%FEV_1$ ≤85% suggested that, whilst CLD was a

significant factor in the univariable modelling, in multivariable modelling, gestation at birth and IUGR were significant and CLD was not. Thus, IUGR and low gestational age may be superior to CLD as predictors of lung function deficits in preterm-born school-aged children. The inverse relationship between gestation at birth and lung function deficits has previously been described (Fawke et al. 2010). In appreciating that birth before 28 weeks' gestation requires respiration to occur at the very beginning of the development of the respiratory units when both epithelial cell differentiation and development of the capillary networks are occurring, it is unsurprising that structural alterations which impact on future respiratory function occurs at a higher incidence.

The association between IUGR and childhood spirometry has also been previously documented. Kotecha et al described how school-aged children with a history of IUGR had greater spirometry deficits compared with counterparts born with appropriate birthweight (Kotecha et al. 2010). Maternal antenatal smoking is known to be a contributor to the development of IUGR (Horta et al. 1997). It is interesting that the PTC group had the highest reported maternal antenatal and postnatal smoking. This is contrary to the increased incidence of IUGR rate found in the P_{low} group. There may be alternative causes of IUGR such as impaired placental function that are responsible for the increased incidence in those with lung function deficits. However, the discordance between self-reported antenatal maternal smoking and urine cotinine has been previously reported – with 35% women who denied smoking having a positive urine cotinine (Britton et al. 2004). Thus, the reliability of parent-reported maternal smoking incidence in my study may be limited.

I have shown that children with a history of CLD were born at the earliest gestations and had the highest incidence of IUGR. They also had the lowest birthweights and reported greater respiratory illness in the neonatal and infancy periods. This raises the question of whether CLD is a confounding factor and should be viewed as an indicator of neonatal and infant illness rather than the optimal predictor of lung function deficits in childhood.

Whilst appreciating results are based on exploratory analysis, further in-depth examination of the predictive value of CLD in identifying those at risk for persisting lung function deficits is required.

4.1.4 Lung function deficits in preterm-born children can be classified as either obstructive or non-obstructive

I have accurately identified preterm-born children with lung function deficits and classified

them into obstructive and non-obstructive airway disease using a combination of $\%FEV_1$ and FEV_1/FVC.

Twenty six percent of preterm-born children compared with 16% of term-born controls had a $\%FEV_1 \leq 85\%$. As expected, preterm-born children with a $\%FEV_1 \leq 85\%$ (P_{low}) had significant deficits across all baseline spirometry measures and the greatest response to bronchodilator compared with both control groups. In contrast, the PTC group had comparable spirometry to term-born controls. This suggests that utilising a cut-off of $\%FEV_1 \leq 85\%$ is useful in identifying those with significant lung function deficits.

The P_{low} group were born at a lower gestational age and had a higher incidence of IUGR - early life factors I have identified to be associated with lung function deficits. They reported higher incidence of respiratory illness in infancy, diagnosis of asthma and - whilst not significantly different - they also reported greater incidence of current respiratory symptoms. This suggests that children who develop persistent lung function deficits have a more complicated course in the neonatal period and infancy.

Despite having the largest spirometry increases, post-bronchodilator measures in the P_{low} group remained significantly lower than both control groups. Simpson et al demonstrated that 92% of children born at <32 weeks' had structural abnormalities which correlated with lung function measures (Simpson et al. 2017). It is reasonable to suggest that children in this study - born in the canalicular or saccular period of embryonic lung development - could have some degree of irreversible altered structural lung development which may have a lasting impact on childhood lung function.

Twenty three percent of the P_{low} group had a positive BDR. These findings are consistent with Vom Hove et al who reported that 25% of preterm-born children with evidence of lung function deficits - irrespective of CLD status - had a positive BDR (Vom Hove et al. 2014). Similarly, in their study of preterm-born children born at $<25^{+6}$ weeks' gestation, Fawke et al also found that 27% of children with abnormal baseline $\%FEV_1$ had a positive BDR (Fawke et al. 2010).

Despite 29% of the P_{low} group having a positive BDR, 71% did not. This suggests the possibility of different respiratory phenotypes within the group which need further identification.

Using the FEV_1/FVC ratio to delineate preterm-born children with a $\%FEV_1 \leq 85\%$ into two respiratory phenotypes - obstructive and non-obstructive - I have described how preterm-

born children with evidence of obstructive lung function deficits have the lowest spirometry and the highest proportions of those with a positive BDR. These findings concur with the hypothesis that lung function deficits in preterm-born children can be classified pragmatically as either obstructive or non-obstructive in nature.

Using an FEV_1/FVC ratio of ≤0.8 to identify obstructive lung function deficits in preterm-born children and an FEV_1/FVC ratio of >0.8 to identify non-obstructive lung function deficits I demonstrated that 41% of preterm-born children with a $\%FEV_1$ ≤85% had obstructive lung function deficits and 59% had non-obstructive lung function deficits. The proportion of preterm-born children with obstructive lung function deficits observed is consistent with several prior studies. However, the observed proportion of children with non-obstructive lung function deficits in these studies was much lower. In a population of children born at ≤32 weeks' gestation, Choukroun et al found that 47% of preterm-born children with a baseline $\%FEV_1$<88% had obstructive lung function deficits, whilst they suggest that only 11% had restrictive or mixed lung function deficits (Choukroun et al. 2015). Similar findings in a population born at ≤26 weeks' gestation were described by the EPICure group, with 45% of those with lung function deficits being described as obstructive and 11% as restrictive (Lum et al. 2011).

These earlier and smaller studies used additional lung function testing methods not routinely available in the community setting. In addition to spirometry, both studies used the gold standard estimation of TLC to identify those with restrictive lung function deficits within their respective cohorts (Pellegrino et al. 2005). It is of interest that neither author discusses those who were not ascribed to either obstructive or restrictive patterns of disease. Neither did they identify whether those with obstructive or restrictive lung function deficits gained greatest benefit from inhaled bronchodilator. Lum et al identified that 27% of children had a positive BDR but did not identify whether these children were attributed to either obstructive or restrictive groups, whilst Choukroun et al analysed positive BDR across groups defined by prior CLD status only (Lum et al. 2011; Choukroun et al. 2015).

To my knowledge, this is the first time the FEV_1/FVC ratio has been used to define two respiratory phenotypes in school-aged preterm-born children with a reduced $\%FEV_1$. Limitations of community spirometry testing, especially the inability to measure TLC, meant I could not be more specific about those with restrictive lung function deficits. Dividing the preterm-born population into obstructive and non-obstructive patterns of lung function deficits may have resulted in children with both restrictive and mixed patterns of lung

function deficits being classified as non-obstructive. However, the lower baseline $\%FEV_1$ and $\%FEF_{25-75\%}$ observed in the obstructive group and lower %FVC in the non-obstructive group suggest that this binary separation is reasonable. It is also reasonable to suggest that further enhanced lung function testing in the non-obstructive group will help differentiate between those with restrictive and mixed lung function deficits.

Regression modelling identified that early life factors associated with each respiratory phenotype were also different. The non-obstructive phenotype was only associated with low gestation at birth as opposed to the obstructive phenotype, which was associated with low gestation at birth, IUGR, CLD and NEC.

The association between low gestation at birth and childhood lung function deficits is well established (Fawke et al. 2010; Simpson et al. 2017). Therefore, it is no surprise that it is a significant factor for both respiratory phenotypes.

The observed association between IUGR and greater response to bronchodilator in the obstructive group is consistent with the "Generation R" study which demonstrated a link between IUGR, airway remodeling and increased airway resistance (Sonnenschein-van der Voort et al. 2016). Altered growth patterns of the fetus have been associated with altered structural development of the lung and potential for dysgenesis (Pike et al. 2012; Duke et al. 2018). Furthermore, with the suggestion of immune system maladaptation in preterm-born children, it is reasonable to suspect that the inflammation linked with CLD and NEC may well be a contributing factor to the development of obstructive lung function deficits (Kunzmann et al. 2013).

The observed differences in early life factors and lung function measures between the two respiratory phenotypes support the assertion that the FEV_1/FVC ratio can be used to identify the two different respiratory phenotypes. It is also reasonable to suggest that the underlying respiratory pathophysiology and subsequent response to inhaled medication associated with each phenotype is likely to be different between the two groups.

Interestingly, having previously questioned the predictive value of CLD, modelling of early life factors in both the obstructive and non-obstructive phenotypes revealed that children with a history of CLD who continue to have lung function deficits are 5 times more likely to have obstructive patterns of disease, suggesting a possible third respiratory phenotype. The association between CLD and obstructive patterns of lung function deficits in childhood has been previously described (Broström et al. 2010; Thunqvist et al. 2018). Additional evidence

also suggests that lung function progressively worsens with age (Um-Bergström et al. 2017), and persist into adulthood (Gough et al. 2012). In contrast, Hirata et al described progression of obstructive lung function deficits in all ELBW children irrespective of CLD status (Hirata et al. 2017).

In view of the increased incidence of obstructive lung function deficits in those with CLD, it is disappointing to see that - despite reporting similar incidence of wheeze in the last 12 months - they have significantly lower diagnosis of asthma and use of inhalers compared to those without CLD. Sub-optimal prescribing of inhaled medications in those with CLD despite a diagnosis of asthma has previously been described (Hennessy et al. 2008). There is a need to further understand this phenomenon and ensure appropriate prescribing of medications to optimise respiratory health.

Whilst my data demonstrates an association between CLD and obstructive lung function deficits, there was no association with severity of CLD. Prior studies have described those with the most severe CLD having the highest spirometry deficits and greatest evidence of reversible obstruction (Broström et al. 2010; Um-Bergström et al. 2017). The small numbers in my study may have produced a type 2 error resulting in an underrepresentation of the association between severity of CLD and development of obstructive lung function deficits. Further investigation of the impact of severity of CLD on the development of obstructive lung function deficits is required.

Despite similar proportions of children with obstructive disease demonstrating a positive BDR in those with and without a history of CLD, a higher proportion of children with a FeNO >35ppb were observed in those without CLD. This suggests different mechanisms of obstructive lung function deficits between preterm-born children with and without a history of CLD and further supports the suggestion of the presence of other respiratory phenotypes.

In summary, I have demonstrated that assessing lung function of all preterm-born children by $\%FEV_1$ identifies children with significant lung function deficits, they were born at earlier gestations, had lower birthweight and had higher incidence of IUGR. They also report more respiratory symptoms. Using the FEV_1/FVC, I have shown that preterm-born children with a $\%FEV_1$ ≤85% can be further classified into obstructive and non-obstructive respiratory phenotypes. Comparison of both groups showed differences in spirometry measures and each group was associated with different early life factors. Children with a history of CLD with evidence of ongoing lung function deficits were more likely to have obstructive lung

function deficits. Further understanding of the response to inhaled treatments will help to identify if this delineation is clinically useful.

4.1.5 Preterm-born children with obstructive lung function deficits have a greater response to a single dose of bronchodilator than those with non-obstructive lung function deficits

The obstructive group had lower baseline spirometry and the greatest increases in spirometry measures post-bronchodilator compared with the non-obstructive group. A much higher proportion of children in the obstructive group also had a positive BDR. These findings support the hypothesis that preterm-born children with obstructive lung function deficits have a greater response to 400mcg salbutamol than those with non-obstructive lung function deficits.

Exploration of baseline spirometry measures to identify those most likely to have a positive BDR showed that FEV_1/FVC ratio and $\%FEF_{25-75\%}$ were good tests and $\%FEV_1$ was a fair test.

Whilst it would be reasonable to use FEV_1/FVC as the initial identifier, recruitment of children participating in the study included those with and without known lung function deficits. $\%FEV_1$ has long been viewed as a reliable and reproducible measure to identify air flow limitation (Jat 2013) and is also associated with long-term outcomes. Therefore, I chose to use $\%FEV_1$ as the initial measure to identify those with airflow limitation in the first instance.

The use of $\%FEF_{25-75\%}$ as an independent measure of obstruction has been discouraged by some authors due to its high inter- and intra-subject variability, whilst others suggest it is useful in identifying early lung function deficits (Simon et al. 2010; Quanjer et al. 2012; Eke Gungor et al. 2019). Further evidence suggests a high correlation between $\%FEF_{25-75\%}$ and both $\%FEV_1$ and FEV_1/FVC meaning that $\%FEF_{25-75\%}$ has little to add to the interpretation of spirometry measures (Quanjer 2014a; Boutin et al. 2015; Lukic and Coates 2015). My analysis showed the diagnostic value of $\%FEF_{25-75\%}$ decreased from good in the whole preterm-born population to fair in those with a $\%FEV_1$ ≤85%, thus not adding further to the use of $\%FEV_1$ as the primary outcome measure.

It is of interest to note that an $FEF_{25-75\%}$ of 68.5% as the optimal level to identify those who had a positive BDR is close to the LLN for $\%FEF_{25-75\%}$ in children (67%) (Quanjer 2014a). The additional usefulness of $\%FEF_{25-75\%}$ in this population requires further examination

Further analysis demonstrated that a combination of %FEV_1 and FEV_1/FVC was the optimal method of identifying preterm-born school-aged children with persisting lung function deficits to recognise those most likely to benefit from inhaled bronchodilator. Interestingly, the optimal cut-off value for identifying those most likely to have a positive BDR for %FEV_1 was 85.5% and for FEV_1/FVC was 0.79. These values are very similar to the pragmatically chosen values of 85% and 0.8 used throughout the study to delineate between obstructive and non-obstructive respiratory phenotypes.

The optimal FEV_1/FVC cut-off 0.79 differs from the current NICE guidance of 0.7 in asthmatic children which is based on cut-off values for adults (NICE 2017a). However, it offers high sensitivity and specificity in this group of children who may have different underlying lung pathologies. These findings further strengthen the assertion that lung function deficits in preterm-born children can be classified as either obstructive or non-obstructive in nature, and that those classified as obstructive will have the greatest response to bronchodilator.

Despite increases in spirometry measures post-bronchodilator, the mean %FEV_1 in both groups remained ≤85%, suggesting that whilst bronchodilator inhalation may improve airflow limitation, there may be a proportion of children for whom its effect may be limited. It would be useful to understand whether some of these children have classical asthma.

I have shown that children classified as having obstructive lung function deficits had a greater response to bronchodilator. There is a need to understand whether these children all have classical asthma or another prematurity-associated disease process.

4.1.6 Lung function deficits in preterm-born children are independent of Th2 driven eosinophilic asthma.

There was no evidence of any difference between preterm- and term-born children in either mean FeNO or the proportions of children with a FeNO >35ppb. Thus, supporting the hypothesis that lung function deficits in preterm-born children are independent of Th2 driven eosinophilic asthma.

This finding is consistent with previous studies and suggests that the cause of prematurity-associated lung function deficits differ to Th2 eosinophilic driven inflammation often associated with asthma (Cazzato et al. 2013; Fortuna et al. 2016). However, having demonstrated there may be different respiratory phenotypes in the preterm-born population, I chose to further examine FeNO measurements in specific groups of preterm-

born children. I also examined the relationship between spirometry and FeNO and whether FeNO has any diagnostic value in identifying those most likely to have a positive BDR.

Despite a greater proportion of children with CLD having a $\%FEV_1$ ≤85%, no difference was observed in FeNO levels when the CLD group was compared with the preterm-born and term-born controls. Existing published evidence related to FeNO and CLD is conflicting. Baraldi et al and Nordlund et al describe how preterm-born children with and without CLD have lower FeNO levels compared to term-born children with and without asthma (Baraldi et al. 2005; Nordlund et al. 2017). However, most studies concur with my findings and identify no difference between children with and without CLD compared with term-born controls (Cazzato et al. 2013; Vollsaeter et al. 2013; Course et al. 2019).

Examination of FeNO levels in preterm-born children by current $\%FEV_1$ status did reveal some differences. Whilst there was no evidence of correlation between baseline spirometry and FeNO levels in this group, children with a $\%FEV_1$ ≤85% were observed to have a higher mean FeNO levels. They also had a higher proportion of children with FeNO >35ppb (23%) compared to preterm- and term-born controls. These findings suggest that a small number of preterm-born children with a $\%FEV_1$ ≤85% have ongoing Th2 driven eosinophilic driven inflammation.

Further division of those with a $\%FEV_1$ ≤85% into obstructive and non-obstructive respiratory phenotypes showed 33% of the obstructive group had a FeNO >35ppb. This proportion is higher than the non-obstructive group (16%) and the 11% observed in term-born controls, which is comparable with the reported incidence of childhood asthma in the UK of 10% (Scholes and Mindell 2019). Thus, suggesting that a higher proportion of children with obstructive lung function deficits have ongoing Th2 driven eosinophilic linked inflammation. Correlation analysis supported this assertion. It identified that the only baseline spirometry measure observed to have a significant correlation with FeNO was FEV_1/FVC. However, the effect size was small. Further correlation between FeNO level and change in $\%FEV_1$ in response to bronchodilator was also only observed in the obstructive group.

Whilst 33% of the obstructive group had a FeNO >35ppb, over 53% had a positive BDR. This observation suggests that approximately 60% of reversible air flow limitation in the obstructive group can be attributed to Th2 driven eosinophilic inflammation. Correlation was found between FeNO levels and the change in $\%FEV_1$ post-bronchodilator in those with the obstructive respiratory phenotype. This association was not observed in those with the non-

obstructive respiratory phenotype. This finding also been described in children with allergic asthma, with an optimal cut-off level of 34ppb (Ciprandi et al. 2013). With this knowledge, it is reasonable to suggest that some of these children may have classical asthma but others will have a disease process associated with prematurity.

On further analysis, a higher proportion of children with obstructive lung function deficits without CLD had a FeNO >35ppb compared with those with CLD. With evidence of increased neutrophils and its chemoattractant IL-8 in induced sputum in preterm-born children with CLD, it is reasonable to suggest that alternative inflammatory processes may be a contributing factor in children with obstructive lung function deficits who have a history of CLD (Kotecha et al. 2003; Teig et al. 2012; Chakraborty et al. 2013).

A smaller proportion of those in the non-obstructive group had a positive BDR and FeNO >35ppb. It is of interest that the proportion of these children who use inhaled medications (11%) was very similar to those who exhibited a positive BDR (12%), and the proportion of children diagnosed with asthma (16%) was the same as those observed to have elevated FeNO levels. With a similar level of children reporting wheeze in the last 12 months (22%), it could be suggested that there are a small number of children in the non-obstructive group who have an element of reversible airflow obstruction that could be attributed to asthma. These children require further lung function testing, including assessment of their lung volumes, gas exchange, airways resistance, response to exercise.

The diagnostic value of FeNO to identify those who are likely to have a positive BDR was poor for all groups except the obstructive phenotype, for which it was a fair test with a good level of sensitivity. However, its poor specificity would risk many false positives. The optimal cut-off in this group being 12.5ppb - which is below the 20ppb suggested as evidence of absence of Th2 driven eosinophilic driven inflammation (Dweik 2011). It is, therefore, reasonable to suggest that, whilst FeNO may have an association with those likely to have a positive BDR in an extremely limited group of children, its use as a diagnostic tool requires caution. There is a need to further examine its diagnostic value in the preterm-born population.

4.2 Strengths and limitations

The RHiNO study is a large MRC funded study designed to address various aspects of lung function in preterm-born school-aged children. I have been able to focus on the findings gathered during the community testing phase of the study. This has enabled me to gather robust high-quality data from 739 children (544 preterm- and 195 term-born). The large

number of participants in the study have enabled a study which is well-powered. It also makes it one of the largest studies of its type in preterm-born children.

Additional data collected as part of the RHiNO study will be used to address some of the questions raised by my analysis and inform clinicians about the optimal treatment options for these children, thus improving future respiratory outcomes.

4.2.1 Recruitment

A notable strength of this study is the recruitment of a large number of preterm-born children who have received care in an era where antenatal maternal corticosteroid administration and neonatal installation of exogenous surfactant are routine. The inclusion of term-born controls as a comparison group further strengthens the data and subsequent reported findings. Whilst clear inclusion criteria enabled a consistent approach to recruitment and reduced recruiters biasing participant selection, the nature of self-selection associated with the study design will inevitably lead to a level of self-selection bias.

In a bid to reduce non-responder bias, I used high-quality information leaflets and employed a repeat mailing strategy of participation invites which resulted in a positive response rate of 23% of preterm- and 25% term-born children. Further parental choice of a home visit or appointment at the hospital for testing at a convenient time for each family further ensured recruitment opportunities were maximised and bias reduced. Comparison of demographic details of both preterm- and term-born responders and non-responders showed those who did not respond were from greater areas of deprivation. The link between poor health outcomes and deprivation are well documented (Pillas et al. 2014). It is likely that the enrolment of those from the non-responders' group would only serve to strengthen my findings.

The decision to recruit children born at ≤34 weeks' contrasts with most of the prior research which have limited recruitment to those born at lower gestational ages. However, with emerging evidence of persisting lung function deficits being experienced by those born at later gestations, I believe that it was important to include those born moderately preterm (Kotecha et al. 2012a; Edwards et al. 2015; Thunqvist et al. 2016).

The parity of observed demographic details and lung function testing results between my population of preterm-born recruits and prior published evidence increases confidence in the results being representative of a wider preterm-born population, increasing the

generalisability of findings beyond children born in South Wales.

4.2.2 Data collection and analysis

Use of spirometry in isolation does not identify all lung function abnormalities. However, it is recognised as the lung function test which offers the greatest discrimination with children and represents the most feasible tool for community use (Kirkby et al. 2008; Lum et al. 2011). The application of ATS/ERS guidelines, validated reference ranges (GLI), and robust quality assessment systems of spirometry performance strengthened the internal validity of data collected. Specific training of personnel undertaking the testing, clear protocols requiring staff to initially demonstrate tests and use animations where available, also strengthened data collection and testing success. Ninety-three percent of enrolled children performed acceptable baseline spirometry. This is higher than previous studies which show that 74% of children between 4 and 17 years old performed acceptable spirometry providing assurance of robust data collection (Loeb et al. 2008). Whilst a proportion of children did not provide post-bronchodilator spirometry, proportions were similar in all analysis groups. Thus, reducing the impact of attrition bias on the results. FeNO collection proved to be more difficult for some preterm-born children with 3% more preterm-born children unable to perform FeNO compared to term-born controls. Children with CLD had the greatest proportion of children who could not perform FeNO and therefore, results of the impact of CLD on FeNO levels should be viewed with caution.

Some of the challenges with interpretation of FeNO in the paediatric population have been discussed throughout this thesis. To overcome the challenges associated with non-parametric distribution of FeNO results, the ATS suggested using a cut-off point to represent presence of Th2 driven eosinophilic inflammation and increased likelihood of response to inhaled corticosteroids. The recommended cut-off level was generated by evidence in adult studies into FeNO levels and the presence/absence of eosinophils in the sputum of adults (Shaw et al. 2007). Whilst my results comply with current recommended cut-off levels, it is reasonable to acknowledge that the optimum level is based on moderate quality evidence. Further work is required to evaluate optimal cut-off values for FeNO levels in comparison with eosinophils in sputum in the paediatric population.

The inherent bias in all retrospective cohort studies is recall bias. The use of a validated respiratory questionnaire alongside the review of data in the questionnaire at the time of visit were employed to reduce the impact of missing data and recall bias. Parental recall for

birthweight has been found to be reliable (Shenkin et al. 2017). However, the reliability of parent recall of neonatal health events is less well understood. Examination of medical notes was employed to improve the quality of information related to neonatal events included in the data analysis. Despite this, it is important to recognise the data presented is reliant on the accuracy of these two sources of information.

The definition of CLD has been a hotly debated topic since Northway et al described this respiratory disease in a selected mature population. There have been several suggested definitions of CLD, with new criteria evolving as medical and technical knowledge develops. The most recent criteria for defining CLD was published after this study commenced and includes assessment of chest radiography alongside more extensive accommodation of various forms of respiratory support and oxygen requirement (Higgins et al. 2018). This new method of assessment has been criticised for being over complicated and at risk of leading to incorrect diagnoses (Bancalari et al. 2019; Stoecklin et al. 2019).

Due to the limitations associated with retrospective data collection and the introduction of Higgins et al classification after commencing the study, I used the more established Jobe and Bancalari's classification of CLD. Defined as an oxygen requirement at 28 days of age in all preterm-born children with disease severity determined at 36 weeks' gestation for those ≤32 weeks' gestation and 56 days of age or discharge in those >32 week's gestation (Jobe and Bancalari 2001). This strategy is consistent with several studies examining childhood lung function in similar populations (Broström et al. 2010; Cazzato et al. 2013; Simpson et al. 2017).

Despite a proportion of children born >32 weeks' and ≤34 weeks' gestation having lung function deficits, none had CLD. Due to limitations in available information from medical notes and parent reported information, I chose to combine those with moderate/severe CLD. Whilst limiting the analysis of impact of disease severity on lung function, this strategy has been used in other studies, largely due to the small numbers of children with moderate and severe CLD.

Whilst some studies advocate using 12% increase in $\%FEV_1$ after administration of bronchodilator as evidence of a positive BDR, whether this should be absolute or relative is also unclear. In addition, Tse et al found that an 8% increase was superior to 12% in diagnosing asthma in children (Tse et al. 2013). Therefore, I believe that my choice to use an

absolute increase in $\%FEV_1$ of 10% was a reasonable point for identifying a significant post-bronchodilator increase in $\%FEV_1$.

The use of fixed cut-off points as opposed to LLN for assessing lung function has been criticised on the basis that it leads to greater misdiagnosis of COPD in those who are over 70 (Swanney et al. 2008). However, further adult studies have suggested that fixed FEV_1/FVC ratio was superior to LLN in the diagnosis of adults in early-stage lung disease (Mohamed Hoesein et al. 2011; Hoesterey et al. 2019). In accepting that both strategies have limitations and that there may be an argument for using the LLN in my analysis (Sylvester et al. 2020), the community spirometry testing was designed to identify children who could participate in the second stage of the RHiNO study – the randomised controlled trial of different inhaled therapies (Goulden et al. 2022). Fixed cut-off values were selected above LLN for use in the study as they are easily accessible in a community setting.

Later analysis of the optimal cut-off values for $\%FEV_1$ and FEV_1/FVC ratio for identifying those who were most likely to respond to inhaled bronchodilator were consistent with those pragmatically chosen. Ultimately, I believe this supports my assertion that the chosen cut-off values can be used in community screening to assess lung function, assist in the selection of children who could benefit from inhaled bronchodilator, support referrals for in-depth lung function testing, and enable targeted health promotion.

Whilst acknowledging that the gold standard test for diagnosing restrictive disease requires measurement of TLC, this measurement is unavailable for use in a community setting. Some adult studies have attempted to use spirometry measures, such as FEV_6/FVC as a proxy for TLC (Vandevoorde et al. 2008; D'Aquino et al. 2010). These results are not transferrable to the paediatric population. The choice to use FEV_1/FVC ratio as proxy for TLC in those with a reduced $\%FEV_1$ to delineate between those with obstructive and non-obstructive disease does represent potential for bias. However, my findings suggest that this combination of $\%FEV_1$ and FEV_1/FVC identifies two groups of children with different respiratory symptoms, response to bronchodilator and FeNO levels. Thus, this strategy could be employed in a community setting to identify those with the obstructive and non-obstructive respiratory phenotypes with a view that further, more sophisticated lung function testing, would help to identify those who have mixed lung function deficits.

Whilst findings related to disease severity in those with CLD and analysis of those in phenotype groups help towards improved understanding of lung function deficits in the

preterm-born population, the study was powered for multi-variable regression analysis of early life factors for a %FEV_1 ≤85% in the whole preterm-born population. Thus, there is a need to acknowledge that the small numbers in these analysis groups may have led to a type two error. The preterm-born population continues to be relatively small. There is a need for greater collaboration to ensure larger numbers can be analysed to provide optimal evidence of the existence of respiratory phenotypes in the preterm-born population and their different responses to inhaled medications.

4.3 Importance of thesis findings, implications for practice and future directions for research

There are several key findings in this thesis which require further consideration as they have implications for both clinicians and researchers.

4.3.1 Importance of thesis findings

Preterm-born school-aged children born continue to have lung function deficits compared to term-born school-aged children. Despite administration of maternal antenatal corticosteroids, neonatal exogenous surfactant treatment, and greater use of lung protective ventilation strategies being routine neonatal care, 26% of preterm-born children had significant lung function deficits. There is a need to more accurately identify these children and understand optimal treatments to improve future respiratory health. Failure to do this risks a generation of preterm-born adults developing significant and life-limiting respiratory disease.

Children with CLD continue to have the lowest spirometry measures. However, not all children with CLD had lung function deficits. Indeed, 62% of children with CLD had normal lung function. Conversely, 23% of children without CLD had significant lung function deficits. This suggests that CLD may not be the optimal early life factor for lung function deficits in childhood and that, perhaps, the continued focus on CLD may result in poor understanding of the real nature of lung function deficits in the preterm-born population.

My data showed that IUGR and gestation at birth were superior predictors of lung function deficits in a general population of preterm-born children. There is a need for researchers and clinicians to re-appraise the importance of CLD in lung function beyond the neonatal period.

Using basic spirometry, I have described the presence of at least two respiratory phenotypes – obstructive and non-obstructive. Whilst acknowledging the limitations of this method of

lung function testing, children classified as obstructive reported greater respiratory symptoms, had lower spirometry measures, greater response to bronchodilator and had higher FeNO levels compared to children classified as non-obstructive.

Having questioned the value of CLD as a predictor of lung function deficits in childhood, the observation that children with CLD had a greater risk for obstructive lung function deficits suggests that it may be an important factor for the development of obstructive lung function deficits. The additional observation that this group had lower FeNO levels suggests the underlying mechanisms of lung function deficits in this population are different from those without CLD.

These findings suggest that lung function deficits in the preterm-born population are not homogenous. There is a need to understand the role of respiratory phenotypes, their underlying lung pathologies and whether they are more likely to respond to different treatments.

4.3.2 Implications for clinical practice

Duijts et al suggest that children with CLD require respiratory follow-up and treatment of lung function deficits (Duijts et al. 2020). Having demonstrated that preterm-born children without CLD also have significant lung function deficits, it is reasonable to suggest that this strategy should extend beyond those with CLD to include all preterm-born children.

Spirometry is low-cost easily accessible lung function test which, with adequate training and support, can be reliably implemented in the community setting to identify significant lung function deficits in children. Routine screening/surveillance with community spirometry in the preterm-born children is a cost-effective way of identifying those with significant lung function deficits and can enable clinicians to initiate and monitor treatment.

Results from the RCT part of the RHiNO trial (part 2) have demonstrated that a 3-month treatment with a combination of LABA and ICS significantly improved respiratory function in preterm-born school-aged children (Goulden et al. 2022). The relatively small numbers in this study did not allow further analysis of whether there are specific respiratory phenotypes who are more likely to respond to these treatments. My data suggests that there are at least two respiratory phenotypes who respond differently to 400mcg salbutamol. Further assessment of these respiratory phenotypes in a clinical trial of LABA/ICS is required. In the meantime, it is reasonable to suggest that children identified to have significant lung function

deficits received a 3-month trial of LABA/ICS with further lung function testing to assess for improvements.

4.3.3 Directions for future research

With the knowledge that some preterm-born children continue to have significant lung function deficits, there is a need to further understand who is at greatest risk for lung function deficits and their response to inhaled treatments.

Whilst my exploratory analysis of early life factors suggests that IUGR and low gestation at birth are superior predictors of lung function deficits than CLD, the analysis is limited, and recently published evidence has described different results. In a combined cohort of Australian children born in 1991-2, 1997, and 2005, Doyle et al suggest that moderate/severe CLD is independently associated with lung function deficits at 8 years old (Doyle et al. 2022). However, there were several significant differences between this cohort and mine. Firstly, Doyle et al's cohort was limited to children born between 22 and 27 weeks' gestation thus limiting the ability to assess the effect of gestational age. Only 66% of their cohort received surfactant suggesting that surfactant use was not routine practice. Furthermore, only 7% of their cohort had growth restriction - as opposed to 14% in my cohort leaving their analysis of the impact of IGUR vulnerable to a type 2 error. It is reasonable to suggest that the differences in results reported by Doyle et al is due to their examining a different population of children. Further in-depth analysis to fully understand the impact of early life factors – including CLD – on childhood lung function is required. Thus, improving the ability to target screening towards the most appropriate children.

Goulden et al recognise the need to replicate the RCT part of the RHiNO trial to confirm their findings in a larger preterm-born population (Goulden et al. 2022). However, there is also a need to determine which children gain the greatest benefit of inhaled therapies and how clinicians can identify them. This requires further exploration of the existence of different respiratory phenotypes and their response to inhaled treatments.

There is a need to look at the underlying mechanisms of any observed phenotypes. Examination of the mechanisms of the obstructive and non-obstructive phenotypes described in my study including proteomics, metabolomics and cytokines in blood, urine, exhaled breath and, if possible, BAL is needed.

Using the FEV_1/FVC to separate those with a $\%FEV_1$ ≤85% into obstructive and non-obstructive respiratory phenotypes, I have demonstrated how this delineation identifies children with different respiratory symptoms and lung function measures. The use of FEV_1/FVC >0.8 to delineate non-obstructive respiratory phenotype is not a validated proxy for TLC which is the gold standard measure for diagnosing non-obstructive disease. However, this group had less respiratory symptoms, a smaller proportion with a positive BDR and FeNO >35ppb, compared with those in the obstructive phenotype - suggesting the nature of lung function deficit in this group is different. Further studies into the validity of using FEV_1/FVC as a proxy for TLC would help in developing lung function testing that is accessible to all preterm-born children.

This study has assessed the lung function in a population of preterm- and term-born school-aged children at a set point in time. There is an opportunity for this large cohort of children to participate in further longitudinal assessment of respiratory health to understand the impact of preterm birth on respiratory health in adolescence and adulthood.

Greater collaborative working in the scientific community in response to the COVID pandemic resulted in the production of high-quality evidence, developments of new treatments and adaptations in clinical practice at rapid speed which inevitably saved many lives. There is an opportunity to mitigate the challenges associated with researching outcomes in this relatively small population by sharing existing data from different cohorts of preterm-born children to improve understanding about the nature and management of lung function deficits in the preterm-born population beyond the neonatal period.

4.4 Thesis summary

This thesis has used readily accessible community lung function tests to accurately describe the lung function of a large cohort of school-aged preterm- and term-born children born in the modern era of neonatal care.

I have shown that 26% of preterm-born children born ≤34 weeks' gestation had significant lung function deficits. I described how CLD was not an optimal predictor for childhood lung function deficits in a general population of preterm-born children, but IUGR and gestation at birth were superior. However, I also discovered that lung function deficits observed in children with CLD were more likely to be obstructive in nature. This suggests that, whilst the predictive value of CLD may be limited, it may be more useful in identifying specific respiratory phenotypes. There is a need to reappraise the value of CLD and other early life

factors for childhood lung function.

To my knowledge this is the first occasion when a combination of FEV_1 and FEV_1/FVC has been used to identify preterm-born children with lung function deficits and classify them into different respiratory phenotypes. I believe that this strategy was successful and provides a basis for further research into whether respiratory phenotypes have a different response to treatments. The use of community-based lung function testing in this thesis demonstrates how preterm-born children can be successfully monitored in a cost effective and accessible way to ensure that lung function deficits can be understood, and optimal respiratory health achieved.

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5 References

Abele-Horn, M., Scholz, M., Wolff, C. and Kolben, M. 2000. High-density vaginal Ureaplasma urealyticum colonization as a risk factor for chorioamnionitis and preterm delivery. *Acta Obstet Gynecol Scand* 79(11), pp. 973-978. doi: 10.1080/00016340009169245

Abraham, M. et al. 2017. A systematic review of maternal smoking during pregnancy and fetal measurements with meta-analysis. *PLoS ONE* 12(2), p. e0170946. doi: 10.1371/journal.pone.0170946.

ACOG. 2015. Committee Opinion No. 623: Emergent Therapy for Acute-Onset, Severe Hypertension During Pregnancy and the Postpartum Period. *Obstetrics & Gynecology* 125(2), pp. 521-525. doi: 10.1097/01.AOG.0000460762.59152.d7

Albertine, K. H. et al. 1999. Chronic lung injury in preterm lambs. *Am J Respir Crit Care Med* 159(3), pp. 945-958. doi: 10.1164/ajrccm.159.3.9804027.

Alescio, T. and Cassini, A. 1962. Induction in vitro of tracheal buds by pulmonary mesenchyme grafted on tracheal epithelium. *J Exp Zool* 150, pp. 83-94. doi: 10.1002/jez.1401500202

Alving, K. and Malinovschi, A. 2010. Basic aspects of nitric oxide. *European Respiratory Monograph.* 49, pp. 1-31. doi: 10.1183/1025448x.00028509

Antosova, M., Mokra, D., Pepucha, L., Plevkova, J., Buday, T., Sterusky, M. and Bencova, A. 2017. Physiology of nitric oxide in the respiratory system. *Physiol Res* 66(Suppl 2), pp. S159-s172. doi: 10.33549/physiolres.933673.

Aquino, S. L., Schechter, M. S., Chiles, C., Ablin, D. S., Chipps, B. and Webb, W. R. 1999. High-resolution inspiratory and expiratory CT in older children and adults with bronchopulmonary dysplasia. *American Journal of Roentgenology* 173(4), pp. 963-967. doi: 10.2214/ajr.173.4.10511158.

Asher, M. et al. 1995. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *European Respiratory Journal* 8(3), pp. 483-491.

Aurora, P., Gustafsson, P., Bush, A., Lindblad, A., Oliver, C., Wallis, C. E. and Stocks, J. 2004a. Multiple breath inert gas washout as a measure of ventilation distribution in children with cystic fibrosis. *Thorax* 59(12), pp. 1068-1073. doi: 10.1136/thx.2004.022590.

Aurora, P., Stocks, J., Oliver, C., Saunders, C., Castle, R., Chaziparasidis, G. and Bush, A. 2004b. Quality control for spirometry in preschool children with and without lung disease. *American journal of respiratory and critical care medicine.* 169(10), pp. 1152-1159. doi: doi: 10.1164/rccm.200310-1453OC

Bachurski, C. J., Ross, G. F., Ikegami, M., Kramer, B. W. and Jobe, A. H. 2000. Intra-amniotic endotoxin increases pulmonary surfactant components and induces SP-B processing in fetal sheep. *Am J Physiol (Lung)* 280, pp. L279-285. doi: 10.1152/ajplung.2001.280.2.L279

Bahner, T., Hilgendorff, A, Rudloff, S, Rawer, D, Gortner, L. 2004. 46 Influence of intrauterine growth restriction on gene expression of surfactant associated proteins in preterm mice. *Pediatric Research* 56 (3), p. 472. doi: 0.1203/00006450-200409000-00069

Balany, J. and Bhandari, V. 2015. Understanding the impact of infection, inflammation, and their persistence in the pathogenesis of Bronchopulmonary dysplasia. *Front Med* 2, p. 90. doi: 10.3389/fmed.2015.00090

Balte, P., Karmaus, W., Roberts, G., Kurukulaaratchy, R., Mitchell, F. and Arshad, H. 2016. Relationship between birth weight, maternal smoking during pregnancy and childhood and adolescent lung function: A path analysis. *Respir Med* 121, pp. 13-20. doi: 10.1016/j.rmed.2016.10.010

Bancalari, E., Abdenour, G. E., Feller, R. and Gannon, J. 1979. Bronchopulmonary dysplasia: clinical presentation. *The journal of pediatrics.* 95(5 Pt 2), pp. 819-823. doi: 10.1016/S0022-3476(79)80442-4

Bancalari, E., Claure, N. and Jain, D. 2019. Diagnostic Classification of Bronchopulmonary Dysplasia: A Compromise between Defining Lung Disease versus Long-Term Outcome Prediction. *Am J Respir Crit Care Med* 200(10), pp. 1322-1323. doi: doi: 10.1164/rccm.201906-1130LE

Baraldi, E., Bonetto, G., Zacchello, F. and Filippone, M. 2005. Low Exhaled Nitric Oxide in School-Age Children with Bronchopulmonary Dysplasia and Airflow Limitation. *American Journal of Respiratory and Critical Care Medicine* 171(1), pp. 68-72. doi: 10.1164/rccm.200403-298OC

Barker, D. J., Godfrey, K. M., Fall, C., Osmond, C., Winter, P. D. and Shaheen, S. O. 1991. Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease. *Bmj* 303(6804), pp. 671-675. doi: 10.1136/bmj.303.6804.671

Barker, D. J. 2004. The developmental origins of adult disease. *J Am Coll Nutr* 23(6 Suppl), pp. 588s-595s. doi: 10.1111/j.1365-2796.2007.01809.x.

Barton, L., Hodgman, J. E. and Pavlova, Z. 1999. Causes of death in the extremely low birth weight infant. *Pediatrics* 103(2), pp. 446-451. doi: 10.1542/peds.103.2.446

Been, J. V., Rours, I. G., Kornelisse, R. F., Jonkers, F., de Krijger, R. R. and Zimmermann, L. J. 2010. Chorioamnionitis alters the response to surfactant in preterm infants. *J Pediatr* 156(1), pp. 10-15. doi: 10.1016/j.jpeds.2009.07.044

Been, J. V., Lugtenberg, M. J., Smets, E., van Schayck, C. P., Kramer, B. W., Mommers, M. and Sheikh, A. 2014. Preterm birth and childhood wheezing disorders: a systematic review and meta-analysis. *PLoS Med* 11(1), p. e1001596. doi: 10.1371/journal.pmed.1001596

Bentsen, M. H. L., Eriksen, M., Olsen, M. S., Markestad, T. and Halvorsen, T. 2016. Electromagnetic inductance plethysmography is well suited to measure tidal breathing in infants. *ERJ Open Research* 2(4), pp. 00062-02016. doi: 10.1183/23120541.00062-2016

Berard, A., Le Tiec, M. and De Vera, M. A. 2012. Study of the costs and morbidities of late-preterm birth. *Archives of Disease in Childhood Fetal & Neonatal Edition* 97(5), pp. F329-334. doi: 10.1136/fetalneonatal-2011-300969

Bisgaard, H., Loland, L., Holst, K. K. and Pipper, C. B. 2009. Prenatal determinants of neonatal lung function in high-risk newborns. *J Allergy Clin Immunol* 123(3), pp. 651-657. doi: 10.1016/j.jaci.2008.11.036

Bjermer, L. et al. 2014. Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med* 108(6), pp. 830-841. doi: 10.1016/j.rmed.2014.02.005

Blencowe, H. et al. 2012. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *The Lancet* 379(9832), pp. 2162-2172. doi: 10.1016/S0140-6736(12)60820-4

Blencowe, H. et al. 2013. Born Too Soon: The global epidemiology of 15 million preterm births. *Reproductive Health* 10(1), pp. 1-14. doi: 10.1186/1742-4755-10-S1-S2

Bokslag, A., van Weissenbruch, M., Mol, B. W. and de Groot, C. J. 2016. Preeclampsia; short and long-term consequences for mother and neonate. *Early Hum Dev* 102, pp. 47-50. doi: 10.1016/j.earlhumdev.2016.09.007

Bolton, C. E., Bush, A., Hurst, J. R., Kotecha, S. and McGarvey, L. 2015. Lung consequences in adults born prematurely. *Thorax* 70(6), pp. 574-580. doi: 10.1136/postgradmedj-2014-206590rep

Bonet, M., Smith, L. K., Pilkington, H., Draper, E. S. and Zeitlin, J. 2013. Neighbourhood deprivation and very preterm birth in an English and French cohort. *BMC Pregnancy Childbirth* 13, p. 97. doi: 10.1186/1471-2393-13-97

Boutin, B. et al. 2015. Forced expiratory flows' contribution to lung function interpretation in schoolchildren. *Eur Respir J* 45(1), pp. 107-115. doi: 10.1183/09031936.00062814

Britton, G. R., Brinthaupt, J., Stehle, J. M. and James, G. D. 2004. Comparison of self-reported smoking and urinary cotinine levels in a rural pregnant population. *J Obstet Gynecol Neonatal Nurs* 33(3), pp. 306-311. doi: 10.1177/0884217504264866

Broström, E. B., Thunqvist, P., Adenfelt, G., Borling, E. and Katz-Salamon, M. 2010. Obstructive lung disease in children with mild to severe BPD. *Respiratory Medicine* 104(3), pp. 362-370. doi: 10.1016/j.rmed.2009.10.008

Brunt, H., Barnes, J., Jones, S. J., Longhurst, J. W. S., Scally, G. and Hayes, E. 2017. Air pollution, deprivation and health: understanding relationships to add value to local air quality management policy and practice in Wales, UK. *J Public Health* 39(3), pp. 485-497. doi: 10.1093/pubmed/fdw084

Burke, H. et al. 2012. Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. *Pediatrics* 129(4), pp. 735-744. doi: 10.1542/peds.2011-2196

Burri, P. H. 1984. Fetal and postnatal development of the lung. *Annu Rev Physiol* 46, pp. 617-628. doi: 10.1146/annurev.ph.46.030184.003153

Bush, A. 2021. Impact of early life exposures on respiratory disease. *Paediatric Respiratory Reviews* 40, pp. 24-32. doi: 10.1016/j.prrv.2021.05.006

Caskey, S. et al. 2016. Structural and Functional Lung Impairment in Adult Survivors of Bronchopulmonary Dysplasia. *Ann Am Thorac Soc* 13(8), pp. 1262-1270. doi: 10.1513/AnnalsATS.201509-578OC

Cazzato, S., Ridolfi, L., Bernardi, F., Faldella, G. and Bertelli, L. 2013. Lung function outcome at school age in very low birth weight children. *Pediatric Pulmonology* 48(8), pp. 830-837. doi: 10.1002/ppul.22676

Chakraborty, M., McGreal, E. P., Davies, P. L., Nowell, M. A., Jones, S. and Kotecha, S. 2013. Role of interleukin-6, its receptor and soluble gp130 in chronic lung disease of prematurity. *Neonatology* 104(3), pp. 161-167. doi: 10.1159/000351015

Choukroun, M. L. et al. 2015. Pulmonary outcome and its correlates in school-aged children born with a gestational age < 32 weeks. *Respiratory Medicine* 107(12), pp. 1966-1976. doi: 10.1016/j.rmed.2013.06.020

Ciprandi, G., Tosca, M. A. and Capasso, M. 2013. High Exhaled Nitric Oxide Levels May Predict Bronchial Reversibility in Allergic Children with Asthma or Rhinitis. *Journal of Asthma* 50(1), pp. 33-38. doi: 10.3109/02770903.2012.740119

Coalson, J. J., Winter, V. and deLemos, R. A. 1995. Decreased alveolarization in baboon survivors with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 152(2), pp. 640-646. doi: 10.1164/ajrccm.152.2.7633720

Coalson, J. J., Winter, V. T., Siler-Khodr, T. and Yoder, B. A. 1999. Neonatal chronic lung disease in extremely immature baboons. *Am J Respir Crit Care Med* 160(4), pp. 1333-1346. doi: 10.1164/ajrccm.160.4.9810071

Condò, V., Pugni, L., Fumagalli, M. and Mosca, F. 2003. Endogenous Nitric Oxide Production in the Airways of Preterm and Term Infants. *Neonatology* 83(2), pp. 113-116. doi: 10.1159/000067964

Copland, I. and Post, M. 2004. Lung development and fetal lung growth. *Paediatric Respiratory Reviews* 5, pp. S259-S264. doi: 10.1016/S1526-0542(04)90049-8

Corwin, B. K., Trembath, A. N. and Hibbs, A. M. 2018. Bronchopulmonary dysplasia appropriateness as a surrogate marker for long-term pulmonary outcomes: A Systematic review. *J Neonatal Perinatal Med* 11(2), pp. 121-130. doi: 10.3233/npm-181756

Costeloe, K., Hennessy, E., Gibson, A. T., Marlow, N. and Wilkinson, A. R. 2000. The EPICure study: outcomes to discharge from hospital for infants born at the threshold of viability. *Pediatrics* 106(4), pp. 659-671. doi: 10.1542/peds.106.4.659

Costeloe, K. L., Hennessy, E. M., Haider, S., Stacey, F., Marlow, N. and Draper, E. S. 2012. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). *The BMJ* 345, p. e7976. doi: 10.1136/bmj.e7976

Course, C. W., Kotecha, S. and Kotecha, S. J. 2019. Fractional exhaled nitric oxide in preterm-born subjects: A systematic review and meta-analysis. *Pediatr Pulmonol* 54(5), pp. 595-601. doi: 10.1002/ppul.24270

Criee, C. P. et al. 2015. Guide to Spirometry. *Pneumologie* 69(3), pp. 147-164. doi: 10.1055/s-0034-1391345

Cristea, A. I. et al. 2021. Outpatient Respiratory Management of Infants, Children, and Adolescents with Post-Prematurity Respiratory Disease: An Official American Thoracic Society Clinical Practice Guideline. *American Journal of Respiratory and Critical Care Medicine* 204(12), pp. e115-e133. doi: 10.1164/rccm.202110-2269ST

D'Aquino, L. C., Rodrigues, S. C., Barros, J. A., Rubin, A. S., Rosario Filho, N. A. and Pereira, C. A. 2010. Predicting reduced TLC in patients with low FVC and a normal or elevated FEV1/FVC ratio. *J Bras Pneumol* 36(4), pp. 460-467. doi: 10.1590/s1806-37132010000400011

Davies, G. and Reid, L. 1970. Growth of the alveoli and pulmonary arteries in childhood. *Thorax* 25(6), pp. 669-681. doi: 10.1136/thx.25.6.669

De Queiroz Andrade, E., Da Silva Sena, C. R., Collison, A., Murphy, V. E., Gould, G. S., Bonevski, B. and Mattes, J. 2020. Association between active tobacco use during

pregnancy and infant respiratory health: a systematic review and meta-analysis. *BMJ Open* 10(9), pp. e037819-e037819. doi: 10.1136/bmjopen-2020-037819

Deschamps, J., Boucekine, M., Fayol, L., Dubus, J. C., Nauleau, S., Garcia, P. and Boubred, F. 2021. Neighborhood Disadvantage and Early Respiratory Outcomes in Very Preterm Infants with Bronchopulmonary Dysplasia. *J Pediatr* 237, pp. 177-182. doi: 10.1016/j.jpeds.2021.06.061

Dessardo, N. S. et al. 2012. Chorioamnionitis and chronic lung disease of prematurity: a path analysis of causality. *Am J Perinatol* 29(2), pp. 133-140. doi: 10.1055/s-0031-1295654

Dessardo, N. S., Dessardo, S., Mustac, E., Banac, S., Petrovic, O. and Peter, B. 2014. Chronic lung disease of prematurity and early childhood wheezing: is foetal inflammatory response syndrome to blame? *Early Hum Dev* 90(9), pp. 493-499. doi: 10.1016/j.earlhumdev.2014.07.002

Doyle, L. W. 2006. Respiratory function at age 8-9 years in extremely low birthweight/very preterm children born in Victoria in 1991-1992. *Pediatr Pulmonol* 41(6), pp. 570-576. doi: 10.1002/ppul.20412

Doyle, L. W., Faber, B., Callanan, C., Freezer, N., Ford, G. W. and Davis, N. M. 2006. Bronchopulmonary dysplasia in very low birth weight subjects and lung function in late adolescence. *Pediatrics* 118(1), pp. 108–113. doi: 10.1542/peds.2005-2522

Doyle, L. W., Adams, A. M., Robertson, C., Ranganathan, S., Davis, N. M., Lee, K. J. and Cheong, J. L. 2017. Increasing airway obstruction from 8 to 18 years in extremely preterm/low-birthweight survivors born in the surfactant era. *Thorax* 72(8), pp. 712-719. doi: 10.1136/thoraxjnl-2016-208524

Doyle, L. W. et al. 2019. Expiratory airflow in late adolescence and early adulthood in individuals born very preterm or with very low birthweight compared with controls born at term or with normal birthweight: a meta-analysis of individual participant data. *Lancet Respir Med* 7(8), pp. 677-686. doi: 10.1016/s2213-2600(18)30530-7

Doyle, L. W., Ranganathan, S. and Cheong, J. 2022. Bronchopulmonary dysplasia and expiratory airflow at 8 years in children born extremely preterm in the post-surfactant era. *Thorax*, doi: 10.1136/thoraxjnl-2022-218792

Drysdale, S. B. et al. 2014. Lung function of preterm infants before and after viral infections. *European Journal of Pediatrics* 173(11), pp. 1497-1504. doi: 10.1007/s00431-014-2343-1

Duijts, L. et al. 2020. European Respiratory Society guideline on long-term management of children with bronchopulmonary dysplasia. *European Respiratory Journal* 55(1), p. 1900788. doi: 10.1183/13993003.00788-2019

Duke, J. W., Gladstone, I. M., Sheel, A. W. and Lovering, A. T. 2018. Premature birth affects the degree of airway dysanapsis and mechanical ventilatory constraints. *Exp Physiol* 103(2), pp. 261-275. doi: 10.1113/ep086588

Dunnill, M. S. 1962. Postnatal Growth of the Lung. *Thorax* 17(4), pp. 329-333.

Duong-Quy, S. 2019. Clinical Utility Of The Exhaled Nitric Oxide (NO) Measurement With Portable Devices In The Management Of Allergic Airway Inflammation And Asthma. *Journal of asthma and allergy* 12, pp. 331-341. doi: 10.2147/JAA.S190489

Dweik, R. A. et al. 2011. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. *Americal Jounral of Respiratory and Critical Care Medicine* 184(5), pp. 602-615. doi: 10.1164/rccm.912011ST

Edwards, M. O., Kotecha, S. J., Lowe, J., Richards, L., Watkins, W. J. and Kotecha, S. 2015. Early-term birth is a risk factor for wheezing in childhood: A cross-sectional population study. *Journal of Allergy & Clinical Immunology* 136(3), pp. 581-587. doi: 10.1016/j.jaci.2015.05.005

Edwards, M. O., Kotecha, S. J., Lowe, J., Richards, L., Watkins, W. J. and Kotecha, S. 2016. Management of Prematurity-Associated Wheeze and Its Association with Atopy. *PLoS ONE* 11(5), p. e0155695. doi: 10.1371/journal.pone.0155695

Eke Gungor, H., Sahiner, U. M. and Altuner Torun, Y. 2019. Spirometry in children with asthma and/or allergic rhinitis: comparison of FEF25-75% with the standard measures. *Minerva Pediatr* 71(2), pp. 103-109. doi: 10.23736/s0026-4946.16.04267-5

Emery, J. L. and Mithal, A. 1960. The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. *Arch Dis Child* 35(184), pp. 544-547. doi: 10.1136/adc.35.184.544

Engineer, N. and Kumar, S. 2010. Perinatal variables and neonatal outcomes in severely growth restricted preterm fetuses. *Acta Obstet Gynecol Scand* 89(9), pp. 1174-1181. doi: 10.3109/00016349.2010.501370

Engle, W. A. 2006. A Recommendation for the Definition of “Late Preterm” (Near-Term) and the Birth Weight–Gestational Age Classification System. *Seminars in Perinatology* 30(1), pp. 2-7. doi.org/10.1053/j.semperi.2006.01.007

Fawke, J. et al. 2010. Lung function and respiratory symptoms at 11 years in children born extremely preterm: the EPICure study. *Am J Respir Crit Care Med* 182(2), pp. 237-245. doi: 10.1164/rccm.200912-1806OC

Fielding, S. et al. 2019. Change in FEV(1) and Feno Measurements as Predictors of Future Asthma Outcomes in Children. *Chest* 155(2), pp. 331-341. doi: 10.1016/j.chest.2018.10.009

Filippone, M., Sartor, M., Zacchello, F. and Baraldi, E. 2003. Flow limitation in infants with bronchopulmonary dysplasia and respiratory function at school age. *Lancet* 361(9359), pp. 753-754. doi: 10.1016/s0140-6736(03)12633-5

Fletcher, C. and Peto, R. 1977. The natural history of chronic airflow obstruction. *British Medical Journal* 1(6077), pp. 1645-1648. doi: 10.1136/bmj.1.6077.1645

Forno, E., Young, O. M., Kumar, R., Simhan, H. and Celedón, J. C. 2014. Maternal obesity in pregnancy, gestational weight gain, and risk of childhood asthma. *Pediatrics* 134(2), pp. e535-546. doi: 10.1542/peds.2014-0439

Fortuna, M., Carraro, S., Temporin, E., Berardi, M., Zanconato, S. and Salvadori, S. 2016. Mid-childhood lung function in a cohort of children with “new bronchopulmonary dysplasia”. *Pediatr Pulmonol* 51(10), pp. 1057-1064. doi: 10.1002/ppul.23422

Gibbons, J. T. D., Wilson, A. C. and Simpson, S. J. 2020. Predicting Lung Health Trajectories for Survivors of Preterm Birth. *Front Pediatr* 8, p. 318. doi: 10.3389/fped.2020.00318

Gibson, A. M., Reddington, C., McBride, L., Callanan, C., Robertson, C. and Doyle, L. W. 2015. Lung function in adult survivors of very low birth weight, with and without Bronchopulmonary dysplasia. *Pediatr Pulmonol* 50(10), pp. 987-994. doi: 10.1002/ppul.23093

Gochicoa-Rangel, L., Vargas-Dominguez, C., Garcia-Mujica, M. E., Bautista-Bernal, A., Salas-Escamilla, I., Perez-Padilla, R. and Torre-Bouscoulet, L. 2013. Quality of spirometry in 5-to-8-year-old children. *Pediatr Pulmonol* 48(12), pp. 1231-1236. doi: 10.1002/ppul.22765

Goldenberg, R. L., Hauth, J. C. and Andrews, W. W. 2000. Intrauterine infection and preterm delivery. *N Engl J Med* 342(20), pp. 1500-1507. doi: 10.1056/nejm200005183422007

Goldenberg, R. L. et al. 2012. The preterm birth syndrome: issues to consider in creating a classification system. *American Journal of Obstetrics and Gynecology* 206(2), pp. 113-118. doi: 10.1016/j.ajog.2011.10.865

Gough, A., Spence, D., Linden, M., Halliday, H. L. and McGarvey, L. P. A. 2012. General and Respiratory Health Outcomes in Adult Survivors of Bronchopulmonary Dysplasia: A Systematic Review. *Chest* 141(6), pp. 1554-1567. doi: 10.1378/chest.11-1306

Goulden, N. et al. 2022. Inhaled Corticosteroids Alone and in Combination With Long-Acting β2 Receptor Agonists to Treat Reduced Lung Function in Preterm-Born Children: A Randomized Clinical Trial. *JAMA pediatrics* 176(2), pp. 133-141. doi: 10.1001/jamapediatrics.2021.5111

Graham, B. L. et al. 2019. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *American Journal of Respiratory and Critical Care Medicine* 200(8), pp. e70-e88. doi: 10.1164/rccm.201908-1590ST

Green, M., Mead, J. and Turner, J. M. 1974. Variability of maximum expiratory flow-volume curves. *J Appl Physiol* 37(1), pp. 67-74. doi: 10.1152/jappl.1974.37.1.67

Hacking, D. F., Gibson, A. M., Robertson, C. and Doyle, L. W. 2013. Respiratory function at age 8-9 after extremely low birthweight or preterm birth in Victoria in 1997. *Pediatr Pulmonol* 48(5), pp. 449-455. doi: 10.1002/ppul.22619

Halvorsen, T., Skadberg, B. T., Eide, G. E., Roksund, O. D., Carlsen, K. H. and Bakke, P. 2004. Pulmonary outcome in adolescents of extreme preterm birth: a regional cohort study. *Acta Paediatr* 93(10), pp. 1294-1300. doi: 10.1111/j.1651-2227.2004.tb02926.x

Halvorsen, T., Skadberg, B. T., Eide, G. E., Roksund, O. D. and Markestad, T. 2006. Better care of immature infants; has it influenced long-term pulmonary outcome? *Acta Paediatr* 95(5), pp. 547-554. doi: 10.1080/08035250500477529

Harding, R. and Maritz, G. 2012. Maternal and fetal origins of lung disease in adulthood. *Semin Fetal Neonatal Med* 17(2), pp. 67-72. doi: 10.1016/j.siny.2012.01.005

Harris, C., Lunt, A., Bisquera, A., Peacock, J. and Greenough, A. 2020. Lung function and exercise capacity in prematurely born young people. *Pediatric Pulmonology* 55(9), pp. 2289-2295. doi: 10.1002/ppul.24918

Health and Safety at Work Act 1974. Available at:https://www.hse.gov.uk/legislatoin/hswa/htm. [Accessed 21st June 2016]

Hennessy, E. M. et al. 2008. Respiratory health in pre-school and school age children following extremely preterm birth. *Archives of Disease in Childhood* 93(12), pp. 1037-1043. doi: 10.1136/adc.2008.140830

Higgins, R. D. et al. 2018. Bronchopulmonary Dysplasia: Executive Summary of a Workshop. *J Pediatr* 197, pp. 300-308. doi: 10.1016/j.jpeds.2018.01.043

Hirata, K., Nishihara, M., Kimura, T., Shiraishi, J., Hirano, S., Kitajima, H. and Fujimura, M. 2017. Longitudinal impairment of lung function in school-age children

with extremely low birth weights. *Pediatr Pulmonol* 52(6), pp. 779-786. doi: 10.1002/ppul.23669

Hoesterey, D. et al. 2019. Spirometric indices of early airflow impairment in individuals at risk of developing COPD: Spirometry beyond FEV(1)/FVC. *Respir Med* 156, pp. 58-68. doi: 10.1016/j.rmed.2019.08.004

Horta, B. L., Victora, C. G., Menezes, A. M., Halpern, R. and Barros, F. C. 1997. Low birthweight, preterm births and intrauterine growth retardation in relation to maternal smoking. *Paediatric and Perinatal Epidemiology* 11(2), pp. 140-151. doi: 10.1046/j.1365-3016.1997.d01-17.x

Huang, I., Mak, D., Cheung, P., Abraham, M., Clemens, T. and Turner, S. 2019. A systematic review of associations between maternal exposures during pregnancy other than smoking and antenatal fetal measurements. *Environ Res* 173, pp. 528-538. doi: 10.1016/j.envres.2019.04.005

Iacobelli, S., Bonsante, F. and Robillard, P.-Y. 2017. Comparison of risk factors and perinatal outcomes in early onset and late onset preeclampsia: A cohort based study in Reunion Island. *Journal of Reproductive Immunology* 123, pp. 12-16. doi: 10.1016/j.jri.2017.08.005

Jat, K. R. 2013. Spirometry in children. *Prim Care Respir J* 22(2), pp. 221-229. doi: 10.4104/pcrj.2013.00042

Jensen, E. A. et al. 2019. The Diagnosis of Bronchopulmonary Dysplasia in Very Preterm Infants. An Evidence-based Approach. *Am J Respir Crit Care Med* 200(6), pp. 751-759. doi: 10.1164/rccm.201812-2348OC

Jobe, A. H. 1999. The New BPD: An arrest of lung development. *Pediatr Res* 46(6), p. 641. doi: 10.1203/00006450-199912000-00007

Jobe, A. H. and Bancalari, E. 2001. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 163(7), doi: 10.1164/ajrccm.163.7.2011060

Joshi, S. and Kotecha, S. 2007. Lung growth and development. *Early Hum Dev* 83(12), pp. 789-794. doi: 10.1016/j.earlhumdev.2007.09.007

Joshi, S., Powell, T., Watkins, W. J., Drayton, M., Williams, E. M. and Kotecha, S. 2013. Exercise-Induced Bronchoconstriction in School-Aged Children Who Had Chronic Lung Disease in Infancy. *The Journal of Pediatrics* 162(4), pp. 813-818. doi: 10.1016/j.jpeds.2012.09.040

Khatri, S. B. et al. 2021. Use of Fractional Exhaled Nitric Oxide to Guide the Treatment of Asthma: An Official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med* 204(10), pp. e97-e109. doi: 10.1164/rccm.202109-2093ST

Kim, D. K., Choi, S. H., Yu, J., Yoo, Y., Kim, B. I. and Koh, Y. Y. 2006. Bronchial responsiveness to methacholine and adenosine 5'-monophosphate in preschool children with bronchopulmonary dysplasia. *Pediatric Pulmonology* 41(6), pp. 538-543. doi: 10.1002/ppul.20402

Kirkby, J. et al. 2008. The EPICure study: comparison of pediatric spirometry in community and laboratory settings. *Pediatric Pulmonology* 43(12), pp. 1233-1241. doi: 10.1002/ppul.20950

Kitaoka, H., Burri, P. H. and Weibel, E. R. 1996. Development of the human fetal airway tree: analysis of the numerical density of airway endtips. *Anat Rec* 244(2), pp. 207-213. doi: 10.1002/(sici)1097-0185(199602)244:2

Korevaar, D. A. et al. 2015. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. *Lancet Respir Med* 3(4), pp. 290-300. doi: 10.1016/s2213-2600(15)00050-8

Kotecha, S., Mildner, R. J., Prince, L. R., Vyas, J. R., Currie, A. E., Lawson, R. A. and Whyte, M. K. 2003. The role of neutrophil apoptosis in the resolution of acute lung injury in newborn infants. *Thorax* 58(11), pp. 961-967. doi: 10.1136/thorax.58.11.961

Kotecha, S., Clemm, H., Halvorsen, T. and Kotecha, S. J. 2018. Bronchial hyper-responsiveness in preterm-born subjects: A systematic review and meta-analysis. *Pediatr Allergy Immunol* 29(7), pp. 715-725. doi: 10.1111/pai.12957

Kotecha, S. J., Watkins, W. J., Heron, J., Henderson, J., Dunstan, F. D. and Kotecha, S. 2010. Spirometric lung function in school-age children: effect of intrauterine growth retardation and catch-up growth. *Am J Respir Crit Care Med* 181(9), pp. 969-974. doi: 10.1164/rccm.200906-0897OC

Kotecha, S. J., Dunstan, F. D. and Kotecha, S. 2012a. Long term respiratory outcomes of late preterm-born infants. *Semin Fetal Neonatal Med* 17(2), pp. 77-81. doi: 10.1016/j.siny.2012.01.004

Kotecha, S. J., Watkins, W. J., Paranjothy, S., Dunstan, F. D., Henderson, A. J. and Kotecha, S. 2012b. Effect of late preterm birth on longitudinal lung spirometry in school age children and adolescents. *Thorax* 67(1), pp. 54-61. doi: 10.1136/thoraxjnl-2011-200329

Kotecha, S. J., Edwards, M. O., Watkins, W. J., Henderson, A. J., Paranjothy, S. and Dunstan, F. D. 2013. Effect of preterm birth on later FEV1: a systematic review and meta-analysis. *Thorax* 68, pp. 760-766. doi: 10.1136/thoraxjnl-2012-203079

Kotecha, S. J., Edwards, M. O., Watkins, W. J., Lowe, J., Henderson, A. J. and Kotecha, S. 2015. Effect of Bronchodilators on Forced Expiratory Volume in 1 s in

Preterm-Born Participants Aged 5 and Over: A Systematic Review. *Neonatology* 107(3), pp. 231-240. doi: 10.1159/000371539

Kotecha, S. J., Gallacher, D. J. and Kotecha, S. 2016. The respiratory consequences of early-term birth and delivery by caesarean sections. *Paediatr Respir Rev* 19, pp. 49-55. doi: 10.1016/j.prrv.2015.12.002

Kotecha, S. J., Watkins, W. J., Lowe, J., Granell, R., Henderson, A. J. and Kotecha, S. 2019. Comparison of the Associations of Early-Life Factors on Wheezing Phenotypes in Preterm-Born Children and Term-Born Children. *Am J Epidemiol* 188(3), pp. 527-536. doi: 10.1093/aje/kwy268

Kunzmann, S., Collins, J. J., Kuypers, E. and Kramer, B. W. 2013. Thrown off balance: the effect of antenatal inflammation on the developing lung and immune system. *Am J Obstet Gynecol* 208(6), pp. 429-437. doi: 10.1016/j.ajog.2013.01.008

Lahra, M. M., Beeby, P. J. and Jeffery, H. E. 2008. Maternal versus fetal inflammation and respiratory distress syndrome: a 10 year hospital cohort study. *Archives of Disease in Childhood - Fetal and Neonatal Edition* 94(1), pp. F13-F16. doi: 10.1136/adc.2007.135889

Lahra, M. M., Beeby, P. J. and Jeffery, H. E. 2009. Intrauterine Inflammation, Neonatal Sepsis, and Chronic Lung Disease: A 13-Year Hospital Cohort Study. *Pediatrics* 123(5), p. 1314. doi: 10.1542/peds.2008-0656

Landry, J. S., Croitoru, D., Jin, Y., Schwartzman, K., Benedetti, A. and Menzies, D. 2012. Health care utilization by preterm infants with respiratory complications in Quebec. *Can Respir J* 19(4), pp. 255-260. doi: 10.1155/2012/606507

Landry, J. S., Tremblay, G. M., Li, P. Z., Wong, C., Benedetti, A. and Taivassalo, T. 2016. Lung Function and Bronchial Hyperresponsiveness in Adults Born Prematurely. A Cohort Study. *Ann Am Thorac Soc* 13(1), pp. 17-24. doi: 10.1513/AnnalsATS.201508-553OC

Lange, P. et al. 2015. Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. *N Engl J Med* 373(2), pp. 111-122. doi: 10.1056/NEJMoa1411532

Lardon-Fernandez, M., Uberos, J., Molina-Oya, M. and Narbona-Lopez, E. 2017. Epidemiological factors involved in the development of bronchopulmonary dysplasia in very low birth-weight preterm infants. *Minerva Pediatr* 69(1), pp. 42-49. doi: 10.23736/s0026-4946.16.04215-8

Lawder, R., Whyte, B., Wood, R., Fischbacher, C. and Tappin, D. M. 2019. Impact of maternal smoking on early childhood health: a retrospective cohort linked dataset analysis of 697 003 children born in Scotland 1997-2009. *BMJ Open* 9(3), p. e023213. doi: 10.1136/bmjopen-2018-023213

Liu, L. et al. 2016. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *The Lancet* 388(10063), pp. 3027-3035. doi: 10.1016/S0140-6736(16)31593-8

Lo, J. et al. 2018. Longitudinal assessment of lung function in extremely prematurely born children. *Pediatric Pulmonology* 53(3), pp. 324-331. doi: 10.1002/ppul.23933

Loeb, J. S., Blower, W. C., Feldstein, J. F., Koch, B. A., Munlin, A. L. and Hardie, W. D. 2008. Acceptability and repeatability of spirometry in children using updated ATS/ERS criteria. *Pediatr Pulmonol* 43(10), pp. 1020-1024. doi: 10.1002/ppul.20908

Lowe, J., Watkins, W. J., Edwards, M. O., Spiller, O. B., Jacqz-Aigrain, E., Kotecha, S. J. and Kotecha, S. 2014. Association between pulmonary ureaplasma colonization and bronchopulmonary dysplasia in preterm infants: updated systematic review and meta-analysis. *Pediatric Infectious Disease Journal* 33(7), pp. 697-702. doi: 10.1002/ppul.23920

Lowe, J., Watkins, W. J., Kotecha, S. J. and Kotecha, S. 2016. Physical Activity and Sedentary Behavior in Preterm-Born 7-Year Old Children. *PLoS ONE* 11(5), p. e0155229. doi: 10.1371/journal.pone.0155229

Lowe, J., Kotecha, S. J., Watkins, W. J. and Kotecha, S. 2017. Effect of fetal and infant growth on respiratory symptoms in preterm-born children. *Pediatric Pulmonology* 53(2), pp. 189-196. doi: 10.1002/ppul.23920

Lowe, J. et al. 2020. Study protocol: azithromycin therapy for chronic lung disease of prematurity (AZTEC) - a randomised, placebo-controlled trial of azithromycin for the prevention of chronic lung disease of prematurity in preterm infants. *BMJ Open* 10(10), p. e041528. doi: 10.1136/bmjopen-2020-041528

Lukic, K. Z. and Coates, A. L. 2015. Does the FEF25-75 or the FEF75 have any value in assessing lung disease in children with cystic fibrosis or asthma? *Pediatr Pulmonol* 50(9), pp. 863-868. doi: 10.1002/ppul.23234

Lum, S., Kirkby, J., Welsh, L., Marlow, N., Hennessy, E. and Stocks, J. 2011. Nature and severity of lung function abnormalities in extremely pre-term children at 11 years of age. *European Respiratory Journal* 37(5), pp. 1199-1207. doi: 10.1183/09031936.00071110

Mabanta, C. G., Pryhuber, G. S., Weinberg, G. A. and Phelps, D. L. 2003. Erythromycin for the prevention of chronic lung disease in intubated preterm infants at risk for, or colonized or infected with Ureaplasma urealyticum. *Cochrane Database Syst Rev* 2020(4), doi: 10.1002/14651858.Cd003744

Malmberg, L. P., Mieskonen, S., Pelkonen, A., Kari, A., Sovijarvi, A. R. and Turpeinen, M. 2000. Lung function measured by the oscillometric method in prematurely born children with chronic lung disease. *Eur Respir J* 16, pp. 598-603. doi: 10.1034/j.1399-3003.2000.16d05.x

Marchn, J. 2014. *Flow-volume-loop*. Available at: https://commons.wikimedia.org/wiki/File:Flow-volume-loop.svg [Accessed: 1st March 2021].

Maritz, G. S., Cock, M. L., Louey, S., Suzuki, K. and Harding, R. 2004. Fetal Growth Restriction Has Long-Term Effects on Postnatal Lung Structure in Sheep. *Pediatric Research* 55(2), pp. 287-295. doi: 10.1203/01.PDR.0000106314.99930.65

Mello, R. R., Silva, K. S., Costa, A. M. and Ramos, J. R. 2015. Longitudinal assessment of the lung mechanics of very low birth weight preterm infants with and without bronchopulmonary dysplasia. *Sao Paulo Med J* 133(5), pp. 401-407. doi: 10.1590/1516-3180.2014.00101812

Melody, S. M., Ford, J., Wills, K., Venn, A. and Johnston, F. H. 2019. Maternal exposure to short-to medium-term outdoor air pollution and obstetric and neonatal outcomes: A systematic review. *Environ Pollut* 244, pp. 915-925. doi: 10.1016/j.envpol.2018.10.086

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R. and Coates, A. 2005. Standardisation of spirometry. *Eur Respir J* 26(2), pp. 319-388. doi: 10.1183/09031936.05.00034805

Mohamed Hoesein, F. A., Zanen, P. and Lammers, J. W. 2011. Lower limit of normal or FEV1/FVC < 0.70 in diagnosing COPD: an evidence-based review. *Respir Med* 105(6), pp. 907-915. doi: 10.1016/j.rmed.2011.01.008

Mohammed, S. H., Habtewold, T. D., Birhanu, M. M., Sissay, T. A., Tegegne, B. S., Abuzerr, S. and Esmaillzadeh, A. 2019. Neighbourhood socioeconomic status and overweight/obesity: a systematic review and meta-analysis of epidemiological studies. *BMJ Open* 9(11), p. e028238. doi: 10.1136/bmjopen-2018-028238

Moore, E., Blatt, K., Chen, A., Van Hook, J. and DeFranco, E. A. 2016. Relationship of trimester-specific smoking patterns and risk of preterm birth. *American Journal of Obstetrics and Gynecology* 215(1), pp. 109-e101. doi: 10.1016/j.ajog.2016.01.167

Murray, C., Foden, P., Lowe, L., Durrington, H., Custovic, A. and Simpson, A. 2017. Diagnosis of asthma in symptomatic children based on measures of lung function: an analysis of data from a population-based birth cohort study. *Lancet Child Adolesc Health* 1(2), pp. 114-123. doi: 10.1016/s2352-4642(17)30008-1

Nair, V., Loganathan, P. and Soraisham, A. S. 2014. Azithromycin and other macrolides for prevention of bronchopulmonary dysplasia: a systematic review and meta-analysis. *Neonatology* 106(4), pp. 337-347. doi: 10.1159/000363493

Nakwan, N., Thidarat Ruklerd, T., Perkleang, T. and Taptawee, P. 2022. The levels and correlations of FeNO, blood eosinophils and lung function in well-controlled asthma. *Adv Respir Med*, doi: 10.5603/ARM.a2022.0015

Narang, I., Rosenthal, M., Cremonesini, D., Silverman, M. and Bush, A. 2008. Longitudinal evaluation of airway function 21 years after preterm birth. *Am J Respir Crit Care Med* 178(1), pp. 74-80. doi: 10.1164/rccm.200705-701OC

Narayanan, M. et al. 2013. Catch-up alveolarization in ex-preterm children: evidence from (3)He magnetic resonance. *Am J Respir Crit Care Med* 187(10), pp. 1104-1109. doi: 10.1164/rccm.201210-1850OC

NHSE. 2000. *Patient Group Directions*. Available at: https://webarchive.nationalarchives.gov.uk/20120503185443/http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4012260.pdf: Department of Health. [Accessed: 24th August].

NHSProtect. 2015. *Lone worker estate mapping exercise*. Available at: https://www.networks.nhs.uk/nhs-networks/nhs-lone-worker-protection-service: [Accessed: 22nd April].

NICE. 2014. *Measuring fractional exhaled nitric oxide concentration in asthma: NIOX MINO, NIOX VERO and NObreath. Diagnostics guidance [DG12].* Available at: https://www.nice.org.uk/guidance/dg12: [Accessed: 16th March].

NICE. 2017a. *Asthmas: diagnosis, monitoring and chronic asthma managament*. Available at: https://www.nice.org.uk/Guidance/NG8 [Accessed: 3rd November 2018].

NICE. 2017b. *Patient groups directives overview*. Available at: https://pathways.nice.org.uk/pathways/patient-group-directions: NICE. [Accessed: 24th August].

Nordlund, B., James, A., Ebersjo, C., Hedlin, G. and Brostrom, E. B. 2017. Differences and similarities between bronchopulmonary dysplasia and asthma in schoolchildren. *Pediatr Pulmonol* 52(9), pp. 1179-1186. doi: 10.1002/ppul.23741

Northway, W. H., Rosan, R. C. and Porter, D. Y. 1967. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med* 276(7), pp. 357-368. doi: 10.1056/nejm196702162760701

Nuttall, A. G. L., Velásquez, W., Beardsmore, C. S. and Gaillard, E. A. 2019. Lung clearance index: assessment and utility in children with asthma. *Eur Respir Rev* 28(154), doi: 10.1183/16000617.0046-2019

Ochs, M. et al. 2004. The number of alveoli in the human lung. *Am J Respir Crit Care Med* 169(1), pp. 120-124. doi: 10.1164/rccm.200308-1107OC

Oostveen, E., MacLeod, D., Lorino, H., Farre, R., Hantos, Z. and Desager, K. 2003. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J* 22(6), pp. 1026-1041. doi: 10.1183/09031936.03.00089403

Panasevich, S., Lindgren, C., Kere, J., Wickman, M., Pershagen, G., Nyberg, F. and Melen, E. 2010. Interaction between early maternal smoking and variants in TNF and GSTP1 in childhood wheezing. *Clin Exp Allergy* 40(3), pp. 458-467. doi: 10.1111/j.1365-2222.2010.03452.x

Pansieri, C., Pandolfini, C., Elie, V., Turner, M. A., Kotecha, S., Jacqz-Aigrain, E. and Bonati, M. 2014. Ureaplasma, bronchopulmonary dysplasia, and azithromycin in European neonatal intensive care units: a survey. *Sci Rep* 4, p. 4076. doi: 10.1038/srep04076

Paranjothy, S., Dunstan, F., Watkins, W. J., Hyatt, M., Demmler, J. C., Lyons, R. A. and Fone, D. 2013. Gestational Age, Birth Weight, and Risk of Respiratory Hospital Admission in Childhood. *Pediatrics* 132(6), p. e1562. doi: 10.1542/peds.2013-1737

Park, C. W., Park, J. S., Jun, J. K. and Yoon, B. H. 2015. Mild to Moderate, but Not Minimal or Severe, Acute Histologic Chorioamnionitis or Intra-Amniotic Inflammation Is Associated with a Decrease in Respiratory Distress Syndrome of Preterm Newborns without Fetal Growth Restriction. *Neonatology* 108(2), pp. 115-123. doi: 10.1159/000430766

Pellegrino, R. et al. 2005. Interpretative strategies for lung function tests. *Eur Respir J* 26(5), pp. 948-968. doi: 10.1183/09031936.05.00035205

Peterson-Carmichael, S. L., Rosenfeld, M., Ascher, S. B., Hornik, C. P., Arets, H. G., Davis, S. D. and Hall, G. L. 2014. Survey of clinical infant lung function testing practices. *Pediatr Pulmonol* 49(2), pp. 126-131. doi: 10.1002/ppul.22807

Petsky, H. L., Cates, C. J., Kew, K. M. and Chang, A. B. 2018. Tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils): a systematic review and meta-analysis. *Thorax* 73(12), pp. 1110-1119. doi: 10.1136/thoraxjnl-2018-211540

PHE. 2019. *Health of women before and during pregnancy: health behaviours, risk factors and inequalities.* Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/atta

chment_data/file/844210/Health_of_women_before_and_during_pregnancy_2019.pdf [Accessed: 28th April 2022].

PHW. 2020. *Smoking in Wales (2020)*. Available at: https://phw.nhs.wales/services-and-teams/observatory/data-and-analysis/smoking-in-wales-2020/ [Accessed: 11 Jan 2022].

Pickerd, N., Williams, E. M. and Kotecha, S. 2013. Electromagnetic inductance plethysmography to measure tidal breathing in preterm and term infants. *Pediatric Pulmonology* 48(2), pp. 160-167. doi: 10.1002/ppul.22584

Pike, K., Jane Pillow, J. and Lucas, J. S. 2012. Long term respiratory consequences of intrauterine growth restriction. *Semin Fetal Neonatal Med* 17(2), pp. 92-98. doi: 10.1016/j.siny.2012.01.003

Pillas, D., Marmot, M., Naicker, K., Goldblatt, P., Morrison, J. and Pikhart, H. 2014. Social inequalities in early childhood health and development: a European-wide systematic review. *Pediatr Res* 76(5), pp. 418-424. doi: 10.1038/pr.2014.122

Powell, C. V. E., McNamara, P., Solis, A. and Shaw, N. J. 2002. A parent completed questionnaire to describe the patterns of wheezing and other respiratory symptoms in infants and preschool children. *Archives of Disease in Childhood* 87(5), p. 376. doi: 10.1136/adc.87.5.376

Pramana, I. A. et al. 2011. Respiratory symptoms in preterm infants: burden of disease in the first year of life. *European Journal of Medical Research* 16(5), pp. 223-230. doi: 10.1186/2047-783X-16-5-223

Proietti, E. et al. 2014. Can infant lung function predict respiratory morbidity during the first year of life in preterm infants? *Eur Respir J* 43(6), pp. 1642-1651. doi: 10.1183/09031936.00149213

Quanjer, P. H., Tammeling, G. J., Cotes, J. E., Pedersen, O. F., Peslin, R. and Yernault, J. C. 1993. Lung volumes and forced ventilatory flows. *European Respiratory Journal* 6(Suppl 16), p. 5.

Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L. and Culver, B. H. 2012. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 40, doi: 10.1183/09031936.00080312

Quanjer, P. H. 2014a. Measurement of $FEF_{25–75\%}$ and $FEF_{75\%}$ does not contribute to clinical decision making. *European Respiratory Journal* 43(4), pp. 1051-1058. doi: 10.1183/09031936.00128113

Quanjer, P. H., Stanojevic, S., Cole, T.J., Stocks, J. 2014b. *GLI-2012 Desktop software for individual calculation.* Available at: www.ers-education.org/guidelines/global-

lung-function-initiative/spirometry-tools/desktop-individual-calculator/ [Accessed: 15th October 2016].

RCOG. 2019. *Care of Women Presenting with Suspected Preterm Prelabour Rupture of Membranes from 24+0 Weeks of Gestation (Green-top Guideline No. 73)*. Available at: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg73/ [Accessed: 22nd January 2022].

Robbins, R. A. and Grisham, M. B. 1997. Nitric oxide. *The International Journal of Biochemistry & Cell Biology* 29(6), pp. 857-860. doi: 10.1016/S1357-2725(96)00167-7

Roberts, D., Brown, J., Medley, N. and Dalziel, S. R. 2017. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database of Systematic Reviews* (3), doi: 10.1002/14651858.CD004454.pub3

Robinson, D. et al. 2017. Revisiting Type 2-high and Type 2-low airway inflammation in asthma: current knowledge and therapeutic implications. *Clin Exp Allergy* 47(2), pp. 161-175. doi: 10.1111/cea.12880

Ronkainen, E., Dunder, T., Peltoniemi, O., Kaukola, T., Marttila, R. and Hallman, M. 2015. New BPD predicts lung function at school age: Follow-up study and meta-analysis. *Pediatr Pulmonol* 50(11), pp. 1090-1098. doi: 10.1002/ppul.23153

Ronkainen, E., Dunder, T., Kaukola, T., Marttila, R. and Hallman, M. 2016. Intrauterine growth restriction predicts lower lung function at school age in children born very preterm. *Archives of Disease in Childhood - Fetal and Neonatal Edition* 101(5), p. F412. doi: 10.1136/archdischild-2015-308922

Rosenfeld, M. et al. 2013. An Official American Thoracic Society Workshop Report: Optimal Lung Function Tests for Monitoring Cystic Fibrosis, Bronchopulmonary Dysplasia, and Recurrent Wheezing in Children Less Than 6 Years of Age. *Annals of the American Thoracic Society* 10(2), pp. S1-S11. doi: 10.1513/AnnalsATS.201301-017ST

Rutkowska, M., Hozejowski, R., Helwich, E., Borszewska-Kornacka, M. K. and Gadzinowski, J. 2018. Severe bronchopulmonary dysplasia - incidence and predictive factors in a prospective, multicenter study in very preterm infants with respiratory distress syndrome. *J Matern Fetal Neonatal Med*, pp. 1-7. doi: 10.1080/14767058.2017.1422711

Ryan, R. M. et al. 2019. Respiratory Medications in Infants <29 Weeks during the First Year Postdischarge: The Prematurity and Respiratory Outcomes Program (PROP) Consortium. *The Journal of Pediatrics* 208, pp. 148-155.e143. doi: 10.1016/j.jpeds.2018.12.009

Saigal, S. and Doyle, L. W. 2008. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *The Lancet* 371(9608), pp. 261-269. doi: 10.1016/S0140-6736(08)60136-1

Sakonidou, S. and Dhaliwal, J. 2015. The management of neonatal respiratory distress syndrome in preterm infants (European Consensus Guidelines--2013 update). *Arch Dis Child Educ Pract Ed* 100(5), pp. 257-259. doi: 10.1136/archdischild-2014-306642

Schittny, J. C. 2017. Development of the lung. *Cell and Tissue Research* 367(3), pp. 427-444. doi: 10.1007/s00441-016-2545-0

Scholes, S. and Mindell, J. S. 2019. *Health survey for England 2018 Asthma*. Available at: http://healthsurvey.hscic.gov.uk/media/81643/HSE18-Asthma-rep.pdf [Accessed: 15th July 2020].

ScotPHO. 2021. *Tobacco use: maternal smoking*. Available at: https://www.scotpho.org.uk/behaviour/tobacco-use/data/maternal-smoking?msclkid=25729498c87211ec83f15b876f036db7 [Accessed: 28th April 2022].

Shah, P. S. et al. 2016. Neonatal Outcomes of Very Low Birth Weight and Very Preterm Neonates: An International Comparison. *The Journal of Pediatrics* 177, pp. 144-152.e146. doi: 10.1016/j.jpeds.2016.04.083

Shaw, D. E., Berry, M. A., Thomas, M., Green, R. H., Brightling, C. E., Wardlaw, A. J. and Pavord, I. D. 2007. The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial. *Am J Respir Crit Care Med* 176(3), pp. 231-237. doi: 10.1164/rccm.200610-1427OC

Shenkin, S. D., Zhang, M. G., Der, G., Mathur, S., Mina, T. H. and Reynolds, R. M. 2017. Validity of recalled v. recorded birth weight: a systematic review and meta-analysis. *Journal of Developmental Origins of Health and Disease* 8(2), pp. 137-148. doi: 10.1017/S2040174416000581

Shennan, A. T., Dunn, M. S., Ohlsson, A., Lennox, K. and Hoskins, E. M. 1988. Abnormal pulmonary outcomes in premature infants: prediction from oxygen requirement in the neonatal period. *Pediatrics* 82(4), pp. 527-532. doi: 10.1542/peds.82.4.527

Simon, M. R. et al. 2010. Forced expiratory flow between 25% and 75% of vital capacity and FEV1/forced vital capacity ratio in relation to clinical and physiological parameters in asthmatic children with normal FEV1 values. *J Allergy Clin Immunol* 126(3), pp. 527-534 e521-528. doi: 10.1016/j.jaci.2010.05.016

Simpson, S. J. et al. 2017. Altered lung structure and function in mid-childhood survivors of very preterm birth. *Thorax* 72(8), p. 702. doi: 10.1136/thoraxjnl-2016-209291

Skylogianni, E., Douros, K., Anthracopoulos, M. B. and Fouzas, S. 2016. The Forced Oscillation Technique in Paediatric Respiratory Practice. *Paediatr Respir Rev* 18, pp. 46-51. doi: 10.1016/j.prrv.2015.11.001

Sly, P., Blake, T. and Islam, Z. 2021. Impact of prenatal and early life environmental exposures on normal human development. *Paediatric Respiratory Reviews* 40, pp. 10-14. doi: 10.1016/j.prrv.2021.05.007

Smith, C., Egunsola, O., Choonara, I., Kotecha, S., Jacqz-Aigrain, E. and Sammons, H. 2015. Use and safety of azithromycin in neonates: a systematic review. *BMJ Open* 5(12), p. e008194. doi: 10.1136/bmjopen-2015-008194

Sonnenschein-van der Voort, A. M., Gaillard, R., de Jongste, J. C., Hofman, A., Jaddoe, V. W. and Duijts, L. 2016. Foetal and infant growth patterns, airway resistance and school-age asthma. *Respirology* 21(4), pp. 674-682. doi: 10.1111/resp.12718

Sørensen, J. K., Buchvald, F., Berg, A. K., Robinson, P. D. and Nielsen, K. G. 2018. Ventilation inhomogeneity and NO and CO diffusing capacity in ex-premature school children. *Respir Med* 140, pp. 94-100. doi: 10.1016/j.rmed.2018.06.006

Stern, D. A., Morgan, W. J., Wright, A. L., Guerra, S. and Martinez, F. D. 2007. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. *The Lancet* 370(9589), pp. 758-764. doi: 10.1016/S0140-6736(07)61379-8

Stocks, J., Hislop, A. and Sonnappa, S. 2013. Early lung development: lifelong effect on respiratory health and disease. *The Lancet Respiratory Medicine* 1(9), pp. 728-742. doi: 10.1016/S2213-2600(13)70118-8

Stoecklin, B., Simpson, S. J. and Pillow, J. J. 2019. Bronchopulmonary dysplasia: Rationale for a pathophysiological rather than treatment based approach to diagnosis. *Paediatric Respiratory Reviews* 32, pp. 91-97. doi: 10.1016/j.prrv.2018.12.002

Strunk, R. C. et al. 2003. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 112(5), pp. 883-892. doi: 10.1016/j.jaci.2003.08.014

Suresh, G. K. and Soll, R. F. 2005. Overview of surfactant replacement trials. *J Perinatol* 25 Suppl 2, pp. S40-44. doi: 10.1038/sj.jp.7211320

Swanney, M. P. et al. 2008. Using the lower limit of normal for the FEV1/FVC ratio reduces the misclassification of airway obstruction. *Thorax* 63(12), pp. 1046-1051. doi: 10.1136/thx.2008.098483

Sweet, D. G. et al. 2013. European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants--2013 update. *Neonatology* 103(4), pp. 353-368. doi: 10.1159/000349928

Sweet, D. G. et al. 2019. European Consensus Guidelines on the Management of Respiratory Distress Syndrome - 2019 Update. *Neonatology* 115(4), pp. 432-450. doi: 10.1159/000499361

Sylvester, K. P. et al. 2020. ARTP statement on pulmonary function testing 2020. *BMJ Open Respir Res* 7(1), p. e000575. doi: 10.1136/bmjresp-2020-000575

Taglauer, E., Abman, S. H. and Keller, R. L. 2018. Recent advances in antenatal factors predisposing to bronchopulmonary dysplasia. *Semin Perinatol* 42(7), pp. 413-424. doi: 10.1053/j.semperi.2018.09.002

Taylor-Robinson, D., Agarwal, U., Diggle, P. J., Platt, M. J., Yoxall, B. and Alfirevic, Z. 2011. Quantifying the impact of deprivation on preterm births: a retrospective cohort study. *PLoS ONE* 6(8), p. e23163. doi: 10.1371/journal.pone.0023163

Teig, N., Allali, M., Rieger, C. and Hamelmann, E. 2012. Inflammatory markers in induced sputum of school children born before 32 completed weeks of gestation. *J Pediatr* 161(6), pp. 1085-1090. doi: 10.1016/j.jpeds.2012.06.007

The Management of Health and Safety at Work (Regulations) 1999. Available at:https://www.legislation.gov.uk/uksi//1999/3242/contents/made. [Accessed 21st June 2016]

Thunqvist, P., Gustafsson, P., Norman, M., Wickman, M. and Hallberg, J. 2015. Lung function at 6 and 18 months after preterm birth in relation to severity of bronchopulmonary dysplasia. *Pediatr Pulmonol* 50(10), pp. 978-986. doi: 10.1002/ppul.23090

Thunqvist, P. et al. 2016. Lung Function at 8 and 16 Years After Moderate-to-Late Preterm Birth: A Prospective Cohort Study. *Pediatrics* 137(4), doi: 10.1542/peds.2015-2056

Thunqvist, P. et al. 2018. Lung function after extremely preterm birth-A population-based cohort study (EXPRESS). *Pediatr Pulmonol* 53(1), pp. 64-72. doi: 10.1002/ppul.23919

Tiwari, A., Rahman, K., Abejie, B., Jain, V. V. and Vempilly, J. J. 2017. Longer duration of asthma is significantly associated with increased RV/TLC ratio. *Respir Med* 124, pp. 44-48. doi: 10.1016/j.rmed.2017.01.011

Townsend, P., Whitehead, M. and Davidson, N. 1992. *Inequalities in Health: The Black Report & the Health Divide (new third edition)*. London: Penguin Books Ltd.

Tse, S. M. et al. 2013. Diagnostic accuracy of the bronchodilator response in children. *The Journal of allergy and clinical immunology* 132(3), pp. 554-559. doi: 10.1016/j.jaci.2013.03.031

Um-Bergström, P. et al. 2017. Lung function development after preterm birth in relation to severity of Bronchopulmonary dysplasia. *BMC Pulmonary Medicine* 17, p. 97. doi: 10.1186/s12890-017-0441-3

Vandevoorde, J., Verbanck, S., Schuermans, D., Broekaert, L., Devroey, D., Kartounian, J. and Vincken, W. 2008. Forced vital capacity and forced expiratory volume in six seconds as predictors of reduced total lung capacity. *Eur Respir J* 31(2), pp. 391-395. doi: 10.1183/09031936.00032307

Vanker, A., Gie, R. P. and Zar, H. J. 2017. The association between environmental tobacco smoke exposure and childhood respiratory disease: a review. *Expert Rev Respir Med* 11(8), pp. 661-673. doi: 10.1080/17476348.2017.1338949

Verheggen, M., Wilson, A. C., Pillow, J. J., Stick, S. M. and Hall, G. L. 2016. Respiratory function and symptoms in young preterm children in the contemporary era. *Pediatr Pulmonol* 51(12), pp. 1347-1355. doi: 10.1002/ppul.23487

Voerman, E. et al. 2019. Association of Gestational Weight Gain With Adverse Maternal and Infant Outcomes. *JAMA* 321(17), pp. 1702-1715. doi: 10.1001/jama.2019.3820

Vogt, B., Falkenberg, C., Weiler, N. and Frerichs, I. 2014. Pulmonary function testing in children and infants. *Physiol Meas* 35(3), pp. R59-90. doi: 10.1088/0967-3334/35/3/R59

Vollsaeter, M., Roksund, O. D., Eide, G. E., Markestad, T. and Halvorsen, T. 2013. Lung function after preterm birth: development from mid-childhood to adulthood. *Thorax* 68(8), pp. 767-776. doi: 10.1136/thoraxjnl-2012-202980

Vollsaeter, M. et al. 2015. Children Born Preterm at the Turn of the Millennium Had Better Lung Function Than Children Born Similarly Preterm in the Early 1990s. *PLoS ONE* 10(12), p. e0144243.

Vom Hove, M., Prenzel, F., Uhlig, H. H. and Robel-Tillig, E. 2014. Pulmonary outcome in former preterm, very low birth weight children with bronchopulmonary dysplasia: a case-control follow-up at school age. *J Pediatr* 164(1), pp. 40-45 e44. doi: 10.1016/j.jpeds.2013.07.045

von Dadelszen, P. and Magee, L. A. 2002. Fall in Mean Arterial Pressure and Fetal Growth Restriction in Pregnancy Hypertension: An Updated Metaregression

Analysis. *Journal of Obstetrics and Gynaecology Canada* 24(12), pp. 941-945. doi: 10.1016/S1701-2163(16)30592-8

WAG. 2019. *National Survey for Wales 2018-19: Adult smoking and e-cigarette use*. Welsh Government. Available at: https://gov.wales/sites/default/files/statistics-and-research/2019-11/adult-smoking-and-e-cigarette-use-national-survey-wales-april-2018-march-2019-437.pdf [Accessed: 2nd Feb 2022].

Walker, I. V. and Cresswell, J. A. 2019. Multiple deprivation and other risk factors for maternal obesity in Portsmouth, UK. *J Public Health* 41(2), pp. 278-286. doi: 10.1093/pubmed/fdy110

Wang, E. E., Ohlsson, A. and Kellner, J. D. 1995. Association of Ureaplasma urealyticum colonization with chronic lung disease of prematurity: results of a metaanalysis. *J Pediatr* 127(4), pp. 640-644. doi: 10.1016/s0022-3476(95)70130-3

Warner, B. B., Stuart, L. A., Papes, R. A. and Wispe, J. R. 1998. Functional and pathological effects of prolonged hyperoxia in neonatal mice. *Am J Physiol* 275(1), pp. L110-L117. doi: 10.1152/ajplung.1998.275.1.L110

Watterberg, K. L., Demers, L. M., Scott, S. M. and Murphy, S. 1996. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. *Pediatrics* 97(2), pp. 210-215. doi: 10.1542/peds.97.2.210

Watterberg, K. L., Scott, S. M. and Naeye, R. L. 1997. Chorioamnionitis, cortisol, and acute lung disease in very low birth weight infants. *Pediatrics* 99(2), p. e6. doi: 10.1542/peds.99.2.e6

Wheeler, K. I., Abdel-Latif, M. E., Davis, P. G., De Paoli, A. G. and Dargaville, P. A. 2015. Surfactant therapy via brief tracheal catheterization in preterm infants with or at risk of respiratory distress syndrome. *Cochrane Database of Systematic Reviews* (5), doi: 10.1002/14651858.CD011672

Wheeler, K. I., Klingenberg, C., Morley, C. J. and Davis, P. G. 2011. Volume-Targeted versus Pressure-Limited Ventilation for Preterm Infants: A Systematic Review and Meta-Analysis. *Neonatology* 100(3), pp. 219-227. doi: 10.1159/000326080

WHO. 2012. *Born Too Soon: The Global action report on preterm Birth.* Geneva: World health organization. Available at: https://apps.who.int/iris/bitstream/handle/10665/44864/9789244503430_rus.pdf [Accessed: 12th March].

Wickham, S., Anwar, E., Barr, B., Law, C. and Taylor-Robinson, D. 2016. Poverty and child health in the UK: using evidence for action. *Archives of Disease in Childhood* 101(8), pp. 759-766. doi: 10.1136/archdischild-2014-306746

Wilding, S. et al. 2019. Are socioeconomic inequalities in the incidence of small-for-gestational-age birth narrowing? Findings from a population-based cohort in the South of England. *BMJ Open* 9(7), p. e026998. doi: 10.1136/bmjopen-2018-026998

Williams, E. M., Pickerd, N., Eriksen, M., Øygarden, K. and Kotecha, S. 2011. Estimation of tidal ventilation in preterm and term newborn infants using electromagnetic inductance plethysmography. *Physiological measurement.* 32(11), pp. 1833-1845. doi: 10.1088/0967-3334/32/11/001

Williams, O., Dimitriou, G., Hannam, S., Rafferty, G. F. and Greenough, A. 2007. Lung function and exhaled nitric oxide levels in infants developing chronic lung disease. *Pediatr Pulmonol* 42(2), pp. 107-113. doi: 10.1002/ppul.20475

Wong, P. M. et al. 2008. Emphysema in young adult survivors of moderate-to-severe bronchopulmonary dysplasia. *Eur Respir J* 32(2), pp. 321-328. doi: 10.1183/09031936.00127107

Xu, Y. P., Hu, J. M., Huang, Y. Q. and Shi, L. P. 2022. Maternal Ureaplasma exposure during pregnancy and the risk of preterm birth and BPD: a meta-analysis. *Arch Gynecol Obstet*, doi: 10.1007/s00404-022-06491-7

Yammine, S., Schmidt, A., Sutter, O., Fouzas, S., Singer, F., Frey, U. and Latzin, P. 2016. Functional evidence for continued alveolarisation in former preterms at school age? *Eur Respir J* 47(1), pp. 147-155. doi: 10.1183/13993003.00478-2015

6 Appendices

Appendix 1

Publication from this thesis.

Appendix 2

Example of Parent and child information leaflets preterm groups.

Appendix 3

Parent and child information leaflets for term-born controls

Appendix 4

Lone worker Standard Operating Procedure (SOP)

Appendix 5

Patient Group Directive (PGD)

Appendix 6

Adapted ISAAC respiratory questionnaire

Appendix 7

Spirometry quality control (QC) forms

6.1 Appendix 1: Publication from this thesis

Hart K, Cousins M, Watkins W J, Kotecha S J., Henderson A J, Kotecha S (2022) Association of Early Life Factors and Prematurity-AssociatedLung Disease: Prospective Cohort Study, *European Respiratory Journal*, 59(5):2101766. doi: 10.1183/13993003.01766-2021.

6.2 Appendix 2: Example of parent and child information leaflets for preterm-born groups.

6.3 Appendix 3: Parent and child information leaflets for term-born controls

6.4 Appendix 4: Lone worker Standard Operating Procedure (SOP)

6.5 Appendix 5: Patient Group Directive (PGD)

6.6 Appendix 6: Adapted ISAAC respiratory questionnaire

6.7 Appendix 7: Spirometry quality control (QC) forms