Current Challenges in the Respiratory Management of Preterm Infants at a Regional and National Level

By

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Abstract

Preterm birth remains a leading public health challenge and is associated with significant mortality and long-term morbidity. Bronchopulmonary dysplasia (BPD) is one of the most common complications of prematurity and is associated with life-long poor neurodevelopmental and respiratory outcomes. The aims of this thesis were to understand the challenges facing the respiratory management of high-risk preterm infants and explore approaches to overcome these that could potentially improve outcomes.

This thesis composed of five studies linking neonatal respiratory care. Firstly, understanding the recent incidence of BPD and respiratory support used in neonatal units in England and Wales from 2010 to 2017 by conducting a population-based retrospective cohort study using a national neonatal database. The findings demonstrate improving survival but BPD affects 1 in 3 extremely and very preterm infants and is on a rising trend. In parallel, there has been a significant shift from continuous positive airway pressure to high flow respiratory support.

Secondly, assessing the association between early preterm transfer and development of BPD or lung injury by conducting a national retrospective cohort study. This study found no association between early preterm transfer and BPD.

Thirdly, a rodent vibration exposure study to investigate the impact of vibration, as observed during ambulance transportation, on lung. The findings revealed no evidence of lung injury observed after vibration exposure.

The fourth study evaluated the current respiratory monitoring in a local neonatal setting by performing a targeted oxygen saturation quality improvement (QI) project. The QI project found oxygen saturation targeting was achieved 34% of the time and post QI this improved to 43%.

Finally, the fifth study was a feasibility observational study using routinely collected vital signs to early predict respiratory deterioration. The observational study found that only 8.5% of cardiorespiratory adverse events were recorded on manual

nursing documentation compared with electronic monitoring. In addition, a pattern of cardiorespiratory events was associated with the need for mechanical ventilation. In conclusion, the survival of extremely and very preterm infants continues to improve. However, BPD is increasing at a time non-evidence based respiratory management is also increasing. The findings presented suggest the need for exploration of the current modifiable risk factors for BPD, optimisation of respiratory management with better monitoring and delivery of care. These steps, at both a national and local level, could help reverse the worrying trend of increasing BPD and improve the long-term health of this high-risk population.

Acknowledgement

Foremost, all praise and thanks go to God for providing me with the power and endurance through all these years to complete this project.

The presented thesis is the result of my PhD study. Although only my name appeared on the cover of this thesis, a considerable number of individuals have taken part in this huge task.

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Declaration

I hereby declare that the work in this thesis was performed within the Academic Division of Child Health, Obstetrics, and Gynaecology at Nottingham University Hospitals, Queen's Medical Centre campus, between October 2016 and December 2019.

Unless acknowledgement is made in the text, this thesis is my own work that accomplished under the supervision of Dr Don Sharkey and Dr Shalini Ojha.

Up to the best of my knowledge, this thesis is an accurate representation of the work performed within the University of Nottingham.

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Participation and presentation

S Algarni, J Dorling, D Sharkey. Can machine learning identify clinical deterioration in premature infants? M&HS faculty postgraduate research forum, school of Medicine, university of Nottingham. June 2017.

S Algarni, L Shipley, I Bloor, J Dorling, S Ojha, D Sharkey. Vibration model and lung injury. Division of child health, obstetrics & gynaecology, school of medicine, university of Nottingham. March 2018.

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S Algarni, S Ojha, D Sharkey. Neonatal oxygen saturation targeting: quality improvement project for preterm infants. East midland neonatal operational delivery network (respiratory study day), Derby, United Kingdom. September 2019.

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List of abbreviations

Abbreviation	Definition
BP	Blood pressure
BPD	Bronchopulmonary dysplasia
cDNA	Complementary deoxyribonucleic acid
COSHH	Control of substances hazardous to health
CPAP	Continuous positive airway pressure
C-section	Caesarean section
Ct	Cycle threshold
DAMP	Damage associated molecular patterns
FiO_2	Fraction of inspired oxygen
gDNA	genomic deoxyribonucleic acid
H&E	Haematoxylin and eosin
HCPs	Healthcare professionals
HeRO	Heart rate characteristics monitoring
HF	High flow
HR	Heart rate
IL1 β	Interleukin-1β
IQR	Interquartile range
IUGR	Intrauterine growth restriction
IUT	Intrauterine transport
IVH	Intraventricular haemorrhage
LNU	Local neonatal unit
M	Expression stability Value

MCP1	Monocyte chemoattractant protein-1
mRNA	Messenger ribonucleic acid
MV	Mechanical ventilation
NEC	Necrotising enterocolitis
NEW	Newborn early warning system
NFkβ	Nuclear factor kappa β
NHS	National health service
NICU	Neonatal intensive care unit
NNAP	National neonatal audit program
NNRD	National neonatal research database
NOTT	Newborn observation track & trigger
NRTC	No reverse transcriptase control
NTC	No template control
NTS	Neonatal trigger score
PDA	Patent ductus arteriosus
PDSA	Plan – do – study - act
PEWS	Paediatric early warning system
PMA	Postmenstrual age
PRR	Pattern recognition receptor
QI	Quality improvement
qPCR	quantitative polymerase chain reaction
r18S	Ribosomal 18s
RDS	Respiratory distress syndrome
RNA	Ribonucleic acid

ROP	Retinopathy of prematurity
RPL13A	Ribosomal protein L13A
RPS29	Ribosomal protein s29
RR	Respiratory rate
RT-PCR	Real time polymerase chain reaction
SCU	Special care unit
SD	Standard deviation
SPA	Surfactant protein A
SPB	Surfactant protein B
SPC	Surfactant protein C
SPD	Surfactant protein D
SpO_2	Peripheral capillary oxygen saturation
TBP	TATA box binding protein
TGFβ1	Transforming growth factor β
TLR	Toll like receptor
TNFα	Tumour necrosis factor-α
WBV	Whole body vibration
YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation

Glossary

Term	Definition
Preterm infant	Infant born before the completion of 37 weeks of pregnancy.
Very preterm infant	Infant born at gestational age less than 32 weeks.
Extremely preterm infant	Infant born at gestational age less than 28 weeks.
Bronchopulmonary dysplasia	Chronic lung disease of prematurity defined when an infant is 36 weeks corrected gestation and still requiring respiratory support.
Neonatal intensive care unit	Hospital unit designed to look after premature and ill newborn infants.

Chapter 1. Introduction

1.1. Preterm birth

Preterm birth is a major concern in neonatal care. Preterm birth not only impacts the baby and their family, but the need for ongoing continuous care and prolonged hospital stay increase the healthcare cost associated with providing this care.

There are important definitions needed to understand the outcomes for preterm infants. Gestational age is defined as the age from the mother's first day of the last menstrual cycle up to the current date and is often confirmed by first trimester ultrasound [1]. Postmenstrual age (PMA) is defined as the gestational age plus the chronological age after delivery [1]. Preterm infants may be grouped according to their birth weight, and independent of gestational age, as: low birth weight refers to a value less than 2500 g, very low birth weight which less than 1500 g, and extremely low birth weight which less than 1000 g [2].

Preterm birth is defined as infants born before the completion of 37 weeks of pregnancy. Premature delivery is often divided into 3 subcategories based on gestational age, including extremely preterm (<28 weeks), very preterm (28 to <32 weeks), and moderate to late preterm (32 to <37 weeks). Preterm birth increases the risk of death and lifelong complications such as chronic respiratory disease. With decreasing gestational age, there is an increase in mortality, morbidity, and often the need for many months of intensive care [3, 4].

1.2. Preterm infant mortality

In 2010, the number of live births was 135 million around the world, and the average preterm birth rate was 11.1%, or approximately 15 million premature infants and this number continues to increase [3]. In 2017, prematurity accounted for 2.5 million deaths per year and was the leading cause of mortality in children under the age of five [5]. There are around 60,000 premature deliveries every year in the UK [6]. In 2011, there were around 6,000 children under 19 years of age

who died in England and Wales, with preterm births accounting for approximately two thirds of these [7].

In Western Europe, the UK has the second worst neonatal mortality rate, defined as death occurring during the first 28 days of life. The mortality rate is 2.1 per 1000 live births in the early neonatal phase of 0-6 days, 0.7 deaths per 1000 live births at the late neonatal phase of 7-28 days, and 4.9 deaths per 1000 live births at the age of less than five years (Table 1.1) [8]. This high rate of infant mortality in the UK is mostly due to the complications of prematurity [7].

Table 1.1 Early neonatal, late neonatal, & under 5 years mortality rate in Western European Countries in 2013 [8]

Country	Deaths per 1000 live births		
	Early neonatal (0-6 days)	Late neonatal (7-28 days)	Under 5 (0-4 years)
Andorra	1·0	0·4	2.6
Austria	1·7	0.6	4.1
Belgium	1.8	0.6	4.2
Cyprus	1.9	0.7	4.1
Denmark	1.7	0.5	3.8
Finland	1.3	0.4	3.0
France	1.3	0.6	3.7
Germany	1.6	0.5	3.6
Greece	1.6	0.9	4.0
Iceland	0.9	0.4	2.4
Ireland	2.0	0.5	4.6
Israel	1.6	0.6	4.3
Italy	1.7	0.7	3.7
Luxembourg	1.1	0.4	2.8
Malta	3.2	1.0	7.0
Netherlands	1.8	0.5	4.1
Norway	1.2	0.4	3.0
Portugal	1.2	0.5	3.5
Spain	1.3	0.7	3.6
Sweden	1.2	0.3	2.7
Switzerland	2.0	0.5	4.3
UK	2.1	0.7	4.9

According to the Office for National Statistics in 2018, during the first year of life, the infant mortality rate was 3.8 per 1,000 live births compared to lowest ever rate recorded in 2014 with rate of 3.6 per 1,000 live births. The neonatal mortality rate was 2.8 deaths per 1,000 live birth, it has remained the same as in 2017 with prematurity related conditions accounting for almost half of these [9]. This small but significant increase reflects a worrying trend with the reversal of decades of decline in infant mortality (Figure 1.1).

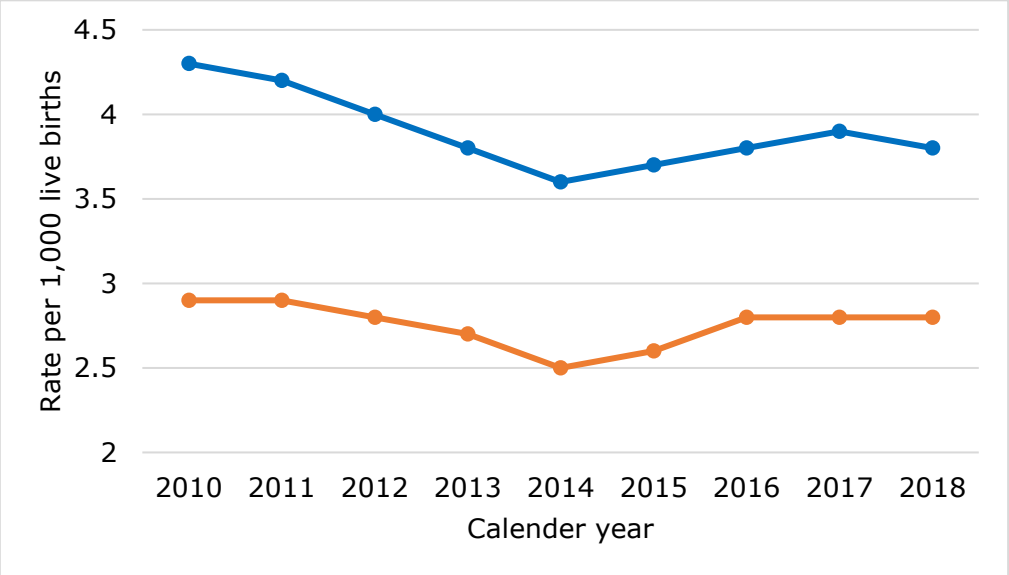


Figure 1.1 Infant and neonatal mortality rate in England and Wales, 2010 to 2018 [9]. Blue line represents infant mortality (defined as death under age of 1 year), and orange line represents neonatal mortality (defined as death under age of 28 days).

The EPICure studies established the survival rate at hospital discharge and significant morbidity for infants born at the gestational age between 20 and 25 weeks. EPICure 1, conducted in UK and Republic of Ireland, included infants between 20 and 25 weeks, for EPICure 2, conducted in England only, the gestation was between 22 and 26 weeks [10]. EPICure 1 (March to December 1995) involved 666 infants and EPICure 2 (January to December 2006) involved 1115 infants [4]. The survival rate at discharge from hospital increased from 40% (n=266) in EPICure 1 to 53% (n=593) in EPICure 2. At 23 gestational weeks, the survival increased by 9.5% between the two cohorts, at 24 gestational weeks by 12%, and

at 25 gestational weeks by 16% [4, 11]. The survival rate at the age of 3 years for infants admitted to the neonatal intensive care unit (NICU) was 39% in 1995 and 52% in 2006 [10].

Furthermore, the mortality rate caused by pulmonary insufficiency decreased from (30%) in 1995 to (16%) in 2006 but it remains as the second principal cause of death of these infants (Table 1.2) [11, 12].

Table 1.2 Principal causes of death of infants admitted for intensive care in those born between 22 and 25 weeks' gestation in England in EPICure and EPICure 2 cohorts [11].

Cause of death	EPICure (n=400)	EPICure 2 (n=522)
Congenital malformation	6 (2)	4 (1)
Pulmonary insufficiency	119 (30)	85 (16) ***
Respiratory distress syndrome/intracerebral haemorrhage/infection	125 (31)	174 (33)
Late sequelae of ventilation	37 (9)	40 (8)
Infection	32 (8)	84 (16) ***
Intracranial haemorrhage	17 (4)	33 (6)
Necrotising enterocolitis	12 (3)	63 (12) ***
Other	42 (11)	35 (7) *
Not known	10 (3)	4 (1) *
Data are number (percentage). *=P<0.05, ***= P<0.001.		

In 2006, the mortality rate decreased in the first week of life and increased after compared with the 1995 data. The increase in reported prematurity related morbidities could have contributed to the overall improvement in mortality rate.

Bronchopulmonary dysplasia (BPD) is a major cause of morbidity in survivors. In EPICure 2, out of 761 infants who survived at 36 weeks PMA and on supplemental oxygen therapy, 39% were classified as moderate and 61% as severe BPD [11]. The development of effective strategies to prevent BPD could further improve overall outcomes of preterm infants.

Berrington *et al* studied the cause of death in northern England of preterm infants over two decades between 1988 and 2008 [13]. There was 680,161 live births during the study period with 1504 deaths. The cause of death was classified into major groups: respiratory, necrotising enterocolitis (NEC), infection, malformations, and other causes. For the analysis, data were grouped into three 7-year epochs and two gestational age subgroups. The three periods were 1988-1994, 1995-2001 and 2002-2008. The two gestational age groups were less than 28 gestational weeks and between 28-31 gestational weeks. The overall mortality rate decreased over the three periods from 19.7%, to 16.7%, and 13.4% respectively. Moreover, the death rate was higher among less than 28 gestational weeks' preterm infants over all three decades. A total of 901 (60%) of the 1,504 deaths were caused by respiratory conditions followed by infection and NEC. Deaths caused by respiratory problems reduced through all gestational age groups and time periods [13].

A similar population study was conducted in the USA to understand the causes of mortality in extremely premature infants during the period 2000 through 2011 [14]. There were 6,075 deaths among 22,248 live births with gestational age ranges between 22 and 28 weeks. The data were again analysed in three epochs from 2000-2003, 2004-2007 and 2008-2011. The overall death rate did not change significantly through the first two periods from 2000-2003 to 2004-2007 but during the periods from 2004-2007 to 2008-2011 this reduced by 9.6% from 285 to 258 deaths per 1000 live births. Respiratory conditions were the second major cause of death after immaturity and accounted for 79 deaths per 1000 live births. The death rate attributed to respiratory conditions was unchanged from 2000-2003 to 2004-2007 (83 and 84 deaths per 1000 live births, respectively), however in 2008-2011 this rate reduced significantly to 68 deaths per 1000 live births [14].

In summary, preterm mortality rate has improved over the last decades. However, late infant mortality remains common, and respiratory disease is one of the leading

causes of infant mortality. The improvement in mortality may be attributed to a number of potential reasons. One of these is the centralisation of neonatal care in the UK that began in 2003.

1.3. Organisation and delivery of neonatal care in England and Wales

The National Neonatal Audit Programme's (NNAP) 2018 annual report, on 2017 data, states there were approximately 750,000 babies born in England, Scotland and Wales and more than 105,000 of these were admitted into a neonatal unit [15].

In 2003, the UK adopted the model of centralised neonatal intensive care, with units specialising in advanced care, surgical procedures and individual therapeutic interventions, resulting in increased demand to transport sick infants to these centres [16]. In the UK, neonatal service provision is based on the organisation of neonatal networks, which is composed of a lead NICU, other NICUs, local neonatal units (LNUs), and special care units (SCUs) within a region and connected via a transport service [17]. This organisation provides equity of access to high standard neonatal care for patients and families; Figure 1.2 shows the UK map of all level 3 NICUs.

Figure 1.2 Neonatal intensive care unit distribution in the UK

There are three categories of neonatal care within the National Health Service (NHS). These three levels include the NICU for complex care, LNUs for high dependency care, and SCUs for initial and short term care[18]. The NHS levels of neonatal care are shown in Table 1.3 [19]. The capacity is planned based on a population of 1 million or more; thus, for intensive care, there is a need for 0.75 cots per 1000 births, 0.7 cots per 1000 births for the high dependency unit, and 4.4 cots per 1000 births for special care [18].

Table 1.3 Categories of UK neonatal care [19]

	Intensive Care (level 3)	High Dependency care (level 2)	Special Care (level 1)
General principle	Care is provided for infants who are critical and have greatest needs in relation to staff skills and staff to patient ratio	Care is provided for infants who require highly skilled staff but the staff patient ratio is less than intensive care unit	Care is provided for infants who require additional care but not at high level
Infants cared for	Those needing invasive positive pressure ventilation, infants weighing <1000g, born at gestational age of <28 weeks, infants who require surgery	Those needing care with apnoeic events, infants receiving continuous positive airway pressure (CPAP), infants receiving parenteral nutrition	Those who need continuous monitoring of their breathing or heart rate, infants on oxygen devices, infants receiving phototherapy

1.4. Normal respiratory physiology of preterm infants

At birth, the lungs must be physiologically prepared to provide oxygen to the newborn infant from the first few breaths. From conception to birth, the fetus lung normally passes through five well-defined stages of lung development: embryonal, pseudoglandular, canalicular, saccular, and alveolar. The fetal lung at 20 weeks has branched completely and all the airways are formed, no air sacs are present, and the capillary beds are forming. By 24 weeks, the lungs are sufficiently mature to allow gas exchange but with the need for surfactant administration, oxygen therapy and assisted ventilation. Table 1.4 and Figure 1.3 summarises the main characteristics for each stage in human lung development [20-22].

Table 1.4 Characteristics of different stages for human lung development [20, 21].

Stage	Duration	Main Characteristics
Embryonal	4 – 7 gestational weeks	• Organogenesis of trachea and major airways • Start formation of two lungs
Pseudoglandular	5 - 17 gestational weeks	• Development of remaining bronchial tree, conducting airways
Canalicular	16 – 26 gestational weeks	• Formation of respiratory bronchioles • Morphogenesis of vascular bed • Appearance of alveolar type I & II cells
Saccular	24 – 38 gestational weeks	• Increase of airspace expansion • Synthesis of surfactant begins
Alveolar	36 gestational weeks – 3 years old	• Maturation of alveolar septa and vascular beds • Alveologenesis

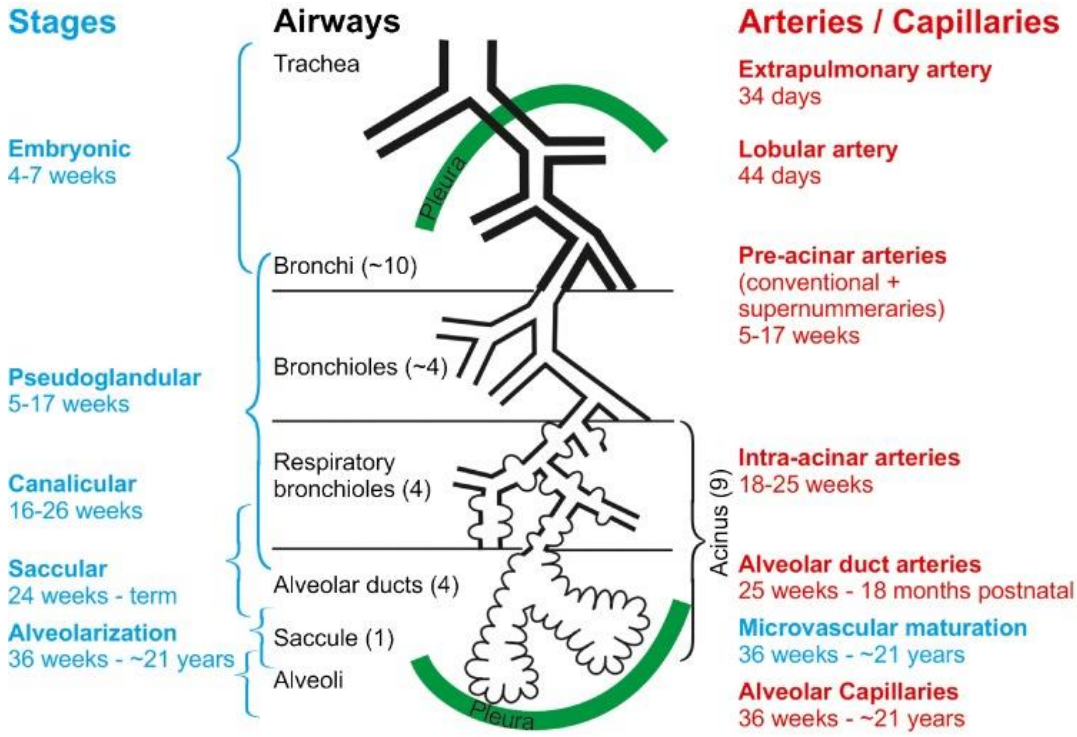


Figure 1.3 Lung development stages (blue) illustrates correlated development of airways (black) and arteries (red). this figure is adapted from Schittney [23]

Preterm birth mainly occurs during the saccular stage and the lungs have not yet developed proper gas exchange structure, leading to the development of respiratory distress syndrome (RDS) [24]. Later on, preterm infants suffer from frequent hospitalisation for respiratory reasons, especially in early life, with the most chronic reason being BPD [25, 26].

1.4.1. Lung surfactants

Surfactant is the major component of the lung responsible for decreasing alveolar surface tension. The surface tension in the lung is defined as a cohesive force of attraction between liquid and gas media, this occurs in lung gas exchange where atmospheric air comes in contact with fluid at the alveolar level. The surfactant forms a thin film layer between these two media and results in a reduction in the surface tension [27].

Pulmonary surfactant is detected in the fetal lung from around 23-30 weeks of gestation [28]. Pulmonary surfactant is a mixture of lipid and protein, and the lipid composes around 90%, with the remaining 10% protein. The surfactant protein part constitutes four types, Surfactant Protein A (SPA), Surfactant Protein B (SPB), Surfactant Protein C (SPC), and Surfactant Protein D (SPD) [28]. SPA is considered the most abundant, followed by SPB, SPC, and SPD. The SPA and SPD proteins are hydrophilic, while SPB and SPC are hydrophobic, where they are important in structural organisation at the air-water interphase [27, 28]. The hydrophilic proteins play a regulatory role in surfactant metabolism and immunological function [27]. SPB deficiency is primarily associated with respiratory failure. In addition, the deficiency of SPB will cause SPC deficiency, which will result in RDS. SPA and SPD are important in pulmonary host defences but they are less important in surfactant processing [28].

Artificial surfactants are a synthetic replacement form of the physiological surfactants, and have been used to treat infants with RDS [29]. The artificial

surfactants have been found to significantly improve preterm outcomes and reduce respiratory complications associated with prematurity, such as BPD [29].

1.5. Respiratory Complications of prematurity

Preterm infants are at risk of developing respiratory complications specific to factors associated with prematurity and medical management [30]. The most common respiratory complication is BPD. This condition is mainly managed in the NICU with infants in hospital for many months.

1.5.1. Bronchopulmonary Dysplasia (BPD)

1.5.1.1. Introduction

BPD is a serious complication unique to preterm infants. In 1960, Northway described BPD as the radiological and clinical abnormalities associated with the presence of RDS that occurred in moderate to late preterm infants [31]. In 1985, O'Brodovich and Mellins revised this with BPD more clearly defined as inflammation, fibrosis, and hypertrophy of the smooth muscles caused by oxidant injury and mechanical ventilation (MV) [32]. Common risk factors leading to BPD are prematurity, oxygen toxicity, and MV [33]. The length of hospital stay for preterm infants diagnosed with BPD is longer than those without BPD [34].

According to latest UK NNAP published in 2019, the incidence of BPD was 37% of preterm infants born at or below 32 weeks of gestational age during the period from 2016 to 2018 [35]. The USA reported that approximately 40% of preterm infants born at or below 28 weeks gestational age developed BPD [36].

1.5.1.2. Definition of BPD

Historically, there was discrepancy in identifying criteria for defining BPD. The earliest definition was based on radiological changes and oxygen requirement only [36]. However, a severity-based definition of BPD considers total duration of supplemental oxygen requirement, need for positive pressure ventilation, and gestational age. For preterm infants less than 32 weeks of gestational age: "Mild

BPD is defined as a need for supplemental oxygen for ≥28 days but not at 36 weeks' PMA or discharge, moderate BPD as oxygen for ≥28 days plus treatment with <30% oxygen at 36 weeks' PMA, and severe BPD as oxygen for ≥28 days plus ≥30% oxygen and/or positive pressure at 36 weeks' PMA" [36, 37]. However, this widely used definition may no longer be valid because it does not account for changes in respiratory support management such as the widespread use of high flow (HF) and incidence of long-term respiratory morbidities for preterm infants [38]. Therefore, a recent revision for this definition was conducted to develop an evidence-based definition. This proposed definition defines BPD based on the respiratory support mode administered at 36 weeks PMA regardless of previous oxygen therapy [39]. BPD severity was ranked as follows: no support = no BPD, nasal cannula ≤ 2 L/min = grade 1 BPD, nasal cannula > 2 L/min or non-invasive ventilation = grade 2 BPD, and invasive ventilation = grade 3 BPD [39].

1.5.1.3. Pathophysiology and risk factors for BPD

The lungs of preterm infants born at early gestations are immature and still undergoing development. Thus, immature lungs do not support efficient gas exchange and any injury of the lung at this stage will affect lung growth and potentially lead to long term pulmonary complications.

The pathogenesis of BPD is multifactorial and risk factors are categorised into either pre or postnatal causes (Figure 1.4). The most significant factor associated with BPD incidence is prematurity [40, 41]. In addition, other factors can contribute to the development of BPD including positive pressure ventilation, oxygen toxicity, and pre/post-neonatal infection. All of these factors can separately or jointly provoke an inflammatory response within the lung [40, 41].

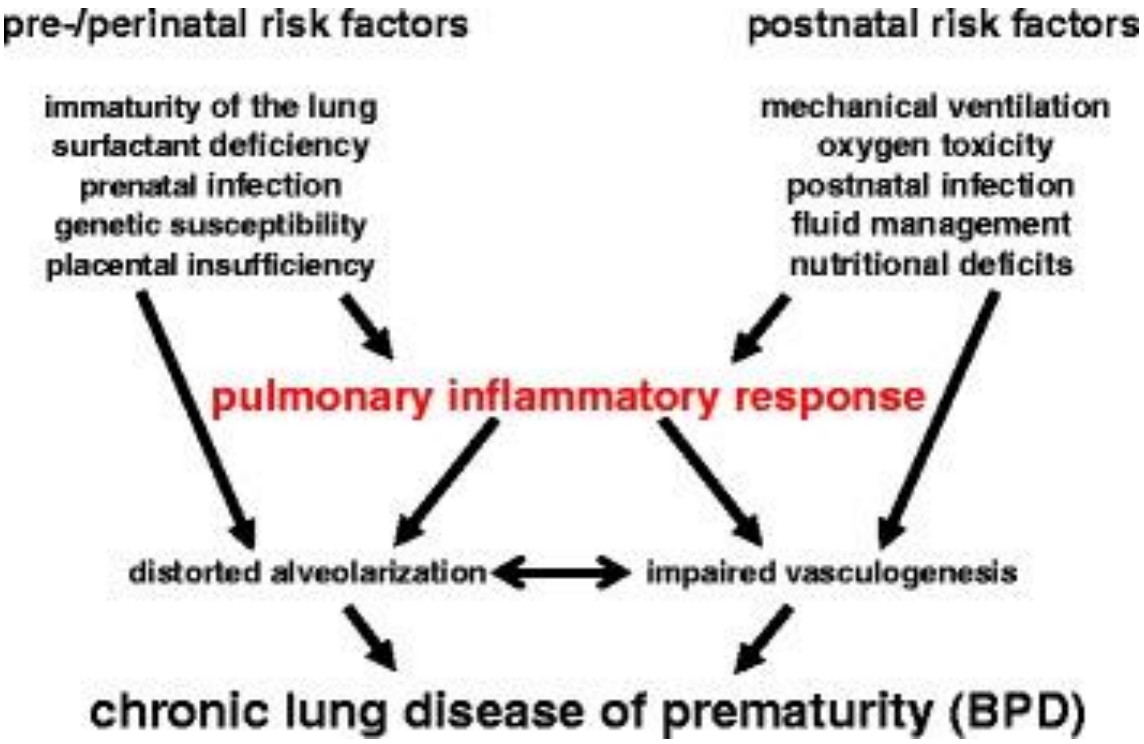


Figure 1.4 Pre/perinatal and postnatal risk factors contributing to the development of BPD. Note: the term chronic lung disease is equivalent to bronchopulmonary dysplasia (BPD) [42].

In normal lung development, alveolarisation starts during late fetal development and continues into the early stage of childhood. In BPD, the alveolarisation is impaired and lung inflammation will be induced by either infectious agents such as viral infection or non-infectious factors such as high oxygen administration and positive pressure ventilation. During the inflammatory response, the release of cytokines and disturbance of growth factor signalling will result in an increase in lung tissue damage and cell apoptosis, which impact all different cell types, and cause inactivation of surfactant [42, 43]. Surfactant inactivation is a key cause of the RDS development in preterm infants [44].

1.5.1.3.1. Prematurity and BPD

In premature infants, BPD is considered the most common cause of respiratory morbidity. In the UK, approximately 37% of premature infants born at or below 32 weeks of gestational age are diagnosed with BPD [35]. BPD incidence is inversely

related to gestational age and birth weight [45]. Therefore, the risk of BPD is greatly increased among extremely preterm and very low birthweight infants [46].

RDS, also known as hyaline membrane disease, is a common cause of NICU admission for preterm infants [47]. Preterm infants have a higher incidence of developing RDS compared to full term infants [48]. The pathogenesis of RDS relates to surfactant deficiency which causes the alveoli to collapse and reduce lung volumes [48]. Infants diagnosed with RDS will show signs of respiratory distress in the first hours of life such as tachypnoea, nasal flaring, grunting, and chest retractions [48]. The use of CPAP is recommended to support an infant with RDS, however, in severe cases the use of positive pressure ventilation is required but this can induce lung injury [48].

1.5.1.3.2. Oxygen toxicity and BPD

The toxic effects that excess oxygen can have on the newborn, particularly if preterm, are well described and known to contribute to significant morbidities such as BPD and retinopathy of prematurity (ROP) [49].

Awareness of the toxicity of oxygen has been increasing in neonatal medicine over the last few decades [50]. Oxidative stress in premature infants occurs due to the free radicals generated by oxygen, which are not handled well by their undeveloped antioxidant systems [51, 52]. Therefore, the imbalance between oxidant and antioxidant factors leads to oxidative stress. Studies conducted by exposing rats and mice to 50% or more oxygen result in the inhibition of pulmonary growth and DNA synthesis [51, 52]. The administration of oxygen can compromise the development of the lung, as high levels of oxygen administration can result in free radical production, injuring the lung and resulting in long-term morphological changes including cessation of cell growth and development [53].

In a trial conducted by Vento, they aimed to reduce the adverse pulmonary outcomes in preterm infants, with the gestational age of 24 to 28 weeks,

resuscitated with the administration of 30% or 90% oxygen at birth [54].They studied urinary markers for oxidative stress which were significantly increased in the group resuscitated using high oxygen concentrations (90%) compared with the group resuscitated with low oxygen (30%). Moreover, the low administered oxygen group had a lower incidence of BPD at hospital discharge compared to high administered oxygen group (15.4% vs 31.7%; $p<0.05$) [54]. However, this study was subjective to a limitation of small study sample specifically for infants below 27 weeks who are more susceptible to developing BPD.

1.5.1.3.3. Mechanical ventilation and BPD

In NICU settings, MV is commonly used as a lifesaving intervention, especially for respiratory failure associated with prematurity. However, the use of positive pressure ventilation and high tidal volumes can cause pulmonary baro/volutrauma, which results in a reduction in lung compliance and the development of lung injury [55]. The need of MV in managing preterm infants has been found to be linked with the development of BPD [56]. In addition, the length of MV correlates with the development of BPD [57]. Therefore, by improving respiratory management practices and avoiding MV, BPD could be reduced [58].

MV affects the dynamic characteristics of the preterm lung, as the decreased compliance caused by surfactant deficiency contributes to non-uniform lung expansion with areas over distended and others collapsed [59]. Therefore, if the premature lung is over inflated via positive pressure ventilation and excess volume, this can lead to cellular injury and inflammation [36].

1.5.1.4. Long-term outcomes of BPD

As the survival rate of preterm infants improve, more survivors will develop BPD [60]. BPD infants suffer from many neurodevelopmental problems; survivors with BPD assessed at preschool and school age were found to perform below average on cognitive, psychomotor, and linguistic evaluation [61].

The literature describes the long-term consequences of BPD as a crucial predictor of long-term respiratory outcomes in child and adulthood. Preterm survivors with BPD were found to have significantly impaired lung function and an increased risk of asthma compared to those without BPD [62, 63]. In addition, Pulmonary diseases developed in adulthood such as asthma and chronic obstructive pulmonary disease have been associated with preterm birth [64].

1.5.1.5. BPD prevention

The improvement in survival rates of preterm infants can increase the burden of BPD among survivors, families, and society [65]. BPD is prevented using evidence-based clinical approaches including prenatal and postnatal management factors as summarised in table 1.5 [66, 67].

Table 1.5 Evidenced based strategies for preventing bronchopulmonary dysplasia (BPD) [66, 67].

Timing of intervention	Intervention
Prenatal	Prevent premature delivery
	Prevent maternal infection
	Prevent maternal smoking in pregnancy
Postnatal	Surfactant therapy
	Postnatal steroid therapy
	Use of continuous positive airway pressure instead of mechanical ventilation
	If ventilated, use of volume targeted instead of pressure targeted ventilation
	Minimise oxygen exposure as possible
	Caffeine therapy
	Vitamin A supplementation

In summary, BPD is mainly the result of lung inflammation in preterm infants and is multifactorial disease. Any advances or new approaches that reduce lung inflammation could help reducing BPD incidence.

The aims of this thesis are to understand the incidence of BPD in England and Wales, current respiratory management and highlight approaches that may improve respiratory outcomes and eventually reduce BPD. The strategies investigated in this thesis are the role of vibration on causing lung injury, minimising oxygen toxicity, and earlier detection of respiratory deterioration.

1.6. Current challenges of preterm respiratory management

1.6.1. Respiratory outcome after preterm inter-hospital transport

1.6.1.1. UK neonatal transport

The centralisation of NICUs specialised in advance care, surgical procedures and special therapeutic interventions has increased the demand to transport critically sick infants to these centres.

With the establishment of NICUs, neonatal mortality has decreased through improved perinatal management and medical practices in the postnatal period [68]. The mortality of newborn infants is inversely proportional to the gestational age [45]. In England, mortality increases when extremely preterm infants are born outside of a tertiary hospital [12]. Nevertheless, the transfer of these infants to tertiary hospitals can improve their survival [69].

The development of antepartum risk assessments in 1960 lead to intrauterine transport (IUT) for high-risk deliveries to high-level neonatal care centres [70]. For very preterm infants, IUT reduces morbidity and mortality compared to postnatal transport [70].

According to the UK Neonatal Transport Group, in the first six months of 2018, there were 7,594 neonatal inter-hospital transports, equating to approximately 15,000 transfers per year [71]. These transfers occur due to one of three main reasons: first, the need for a specialised unit; for example, if the extremely preterm infant needed intensive care treatment. Secondly, babies were transferred to a lower-level unit once they were more stable. Thirdly, due to a lack of cot capacity or staff.

1.6.1.2. Risks encountered during inter-hospital transport

Safe transport is the primary goal during inter-hospital transfer. The most common adverse events occurring during inter-hospital transport for critically ill patients include hypothermia, medication error, tachycardia, loss of intravenous access, and

cyanosis [72]. The most serious and life-threatening adverse events include cardiac arrest, bradycardia, hypotension, and inadequate respiratory support caused by a failed oxygen administration system, mechanical ventilator malfunction, as well as airway events, such as an unplanned extubation [72]. In addition, a well-known clinical risk associated with inter-hospital transport is the increased incidence of intraventricular haemorrhage (IVH) specifically in very low birthweight infants [73].

A prospective study quantified adverse events, occurring during emergency transfer for infants in London between 2009 and 2010 [74]. They found that out of a total of 560 transfers, adverse events were recorded in 261 (52%) cases, being classified as either clinical (n=152) or non-clinical (n=179) events [74]. Over-ventilation was the major clinical adverse event, accounting for 40 events (26%), followed by endotracheal tube malposition, which occurred 35 times (24%) [74]. These factors result in sub-optimal respiratory management and in the preterm infants could lead to the development of lung injury.

1.6.1.3. Vibration encountered during neonatal inter-hospital transport

The inter-hospital transfer of infants uses different modes of transport, such as ambulance, rotary-wing craft, and fixed-wing craft. The aim of each transfer is to be safe and keep the infant stable during transportation. However, specific environmental conditions can affect this goal and compromise the infant's stability; these conditions include excessive noise, mechanical vibration, and environmental temperature [75]. During incubator transportation, the vibration and noise level go beyond the recommended safe limits for adults [76-79], but there are no defined safe vibration levels for infants. These environmental stressors can compromise the physiological and neurological stability , potentially leading to an increase in morbidity [76]. As a result, inter-hospital transfers are conducted by specialised teams who are well-trained and prepared with equipment very similar to NICU settings. Thus, while the level of care provided during transfers remains the same,

it is unclear if these stressors, such as vibration, can contribute to injury and subsequent morbidity [80].

Previously, several studies were conducted to quantify vibration during inter-hospital transfer. In adults, vibration that exceeded 1.5m/s^2 was very uncomfortable, with more than 2m/s^2 considered extremely uncomfortable for the wellbeing of an adult [76]. In 1981, Shenai *et al* found that the mechanical vibration levels during ambulance transport of infants was prolonged, low in frequency, and high in amplitude, between 2 and 6 m/s^2 [75]. In 2011, Karlsson *et al* quantified whole body vibration (WBV) and found that the peak value for WBV during ambulance transport was 2.85 m/s^2 [77]. In 2011, Bouchut *et al* quantified the range of vibration during ground ambulance transportation to be between 0.3 to 0.6 m/s^2 [81]. Recently, in 2017, Blaxter *et al* conducted a study to quantify vibration on neonatal patients during inter-hospital transport. They found that the vibration currently used to secure infants inside incubators exceeded 0.5m/s^2 [80]. At the time of writing, this was the only study that measured the level of vibration at the neonatal head and found that the exposure to be higher than that observed at the incubator level [80]. However, many previous studies quantified vibration at level of incubator wall [77, 81], which could mask the vibration level at the infant's head. Finally, despite the efforts to quantify vibration during neonatal transport, the safe level of vibration for neonates remains unknown.

1.6.1.4. Respiratory outcomes of transported preterm infants

The EPICure 2 study aimed to assess the outcomes for all extremely preterm infants and demonstrated an increase in mortality and morbidity following postnatal inter-hospital transfers [12]. Additionally, a multicentre cohort study conducted in USA to assess the outcomes for outborn extremely preterm infants, who transferred on the day of birth, during the period between 2000 and 2014, and found that outborn preterm infants undergoing transport had a greater risk of BPD compared to inborns [69].

Shlossman *et al* conducted a study to assess the difference in the clinical outcome between preterm infants transported postnatally compared to those undergoing IUT between 1991 and 1994 in the US [82]. A significant increase in the length of NICU stay, extended oxygen exposure and a longer period of MV were found to be associated with postnatal transport of infants, born in the gestation age range between 24-34 weeks, compared to IUT [82]. Furthermore, RDS, BPD, and mortality increased in inter-hospital transports compared to IUT [82].

A Canadian study examined the outcomes of outborn and inborn preterm infants below 32 weeks gestational age and admitted to Canadian neonatal network NICU from 1996 to 1997 [83]. This study reported that outborns have a higher rate of RDS compared to inborn preterm infants, and the BPD rate was significantly increased among outborn preterm infants born with gestational age below 27 weeks [83].

Recently, a retrospective cohort study was conducted using UK National Neonatal Research Database for preterm infants below 32 weeks gestational age to assess the impact of transport on the incidence of IVH , from 2007 to 2016 [84]. This study reported that transported preterm infants are at higher risk of severe IVH and spend more days on MV compared to the inborn infants [84].

These differences between outcomes when comparing transported babies to either non-transported babies or IUT could be related to the quality of antenatal care and delivery room resuscitation/stabilisation in the birth hospital, or postnatal inter-hospital transport conditions. To the best of my knowledge, no study has explored the relationship between inter-hospital transport, as a predictor of BPD development, and BPD incidence. In addition, no previous study examined the impact of the real-life inter-hospital WBV, as occurring during inter-hospital transport, on the development of BPD.

1.6.1.5. Vibration and the respiratory system

A study by Shah and colleagues is the only study to have investigated the potential impact that vibration can have on the neonatal lung. They highlighted the impact of vibration upon the respiratory system by exposing ventilated rat pups to an average vibration impulse of 27.4 m/s^2 [85]. The respiratory system mechanics were found to deteriorate as the airway resistance increased and lung compliance decreased. Furthermore, the lung histology demonstrated increased pulmonary oedema and increased neutrophils in the vibration group compared to the controls. The vibration group also had a reduction in total phospholipid content, while the RT-PCR showed that messenger ribonucleic acid (mRNA) levels for Surfactant Protein A, B, and C were significantly decreased [85].

1.6.2. Oxygen saturation targeting for preterm infants

Aerobic metabolism takes place to generate energy for humans in the presence of an adequate oxygen supply [49]. Oxygen therapy is the most commonly used therapy in the NICU [50]. It is essential for cell respiration and weight gain [86]. Fifty years ago, oxygen had been used widely without any restrictions until a clinical trial demonstrated the association between oxygen therapy and ROP [87].

The optimal oxygen saturation levels needed to improve survival without causing other morbidity for preterm infants remains unknown. Supplemental oxygen targeting a peripheral capillary oxygen Saturation ($SpO_{2)}$ of less than 90% in the first week of life was found to increase the incidence of death, pulmonary vascular resistance and apnoea in extremely preterm infants [88]. Conversely, targeting SpO_2 of more than 90% was found to increase morbidity including ROP and chronic lung disease [88].

1.6.2.1. Monitoring oxygen saturation

The precise evaluation of arterial blood gases is crucial and very important in critical care setting. Arterial blood sampling is an invasive procedure used to evaluate a patient's oxygenation status and requires a blood gas analyser. The need for

developing non-invasive technique to measure the oxygenation status originated in the early 1930s. The first non-invasive oximeter was developed by Matthes using two wavelengths of light in order to compensate for thickness of the tissue and unknown blood content [89].

1.6.2.1.1. Pulse oximetry

Pulse oximetry is a crucial tool in respiratory management as studies have demonstrated skilled healthcare professionals (HCPs) are unable to detect hypoxemia by physical assessment until the oxygen saturation is under 80% [90]. Pulse oximetry depends on two physical principles: the first is the ability of arterial blood to originate a pulsatile signal, this helps to isolate the tissue and venous blood, and the second is the variation between oxyhaemoglobin and deoxyhaemoglobin in terms of light absorption. The oximeter emits red and infrared light, the oxyhaemoglobin absorbs more infrared light and less red light while the reverse is correct for reduced haemoglobin. The ratio of light absorbencies is calibrated based on direct measurements of arterial oxygen saturation in volunteers [91].

Arterial blood gas is the gold standard approach in determining oxygenation status [92]. However, it is difficult and painful to withdraw blood samples from small preterm infants frequently, and it is associated with loss of blood volume and increased transfusion requirements. Therefore, the use of non-invasive, reliable SpO_2 for continuous monitoring of oxygenation is far safe and aids the titration of respiratory support.

1.6.2.2. Optimal SpO2 range for preterm infants

The optimal oxygen saturation target for premature infants is unknown. Several trials have tried to address this with the aim of reducing morbidity and mortality.

The SUPPORT trial was conducted during the period from 2005-2009 [93]. This study aimed to find the appropriate range of oxygen saturation for preterm infants,

which reduces the incidence of ROP with no other adverse outcomes. This study involved 1,316 preterm infants, between 24-28 weeks gestational age, randomised into two groups: 1) low target (85-89%) or 2) high target (91-95%) oxygen saturation range. They found no difference in the ROP incidence between the low or high oxygen saturation group (28.3% and 32.1% respectively). However, this difference was considered clinically important. More importantly, the mortality rate before discharge increased in the low group compared to the high oxygen saturation group (19.9% and 16.2% respectively) [93].

The BOOST-II study involved two trials conducted in Australia and the United Kingdom between 2006 and 2010 [94]. Again, these studies aimed to establish the safest range of oxygen saturation and involved 2,108 infants. Preterm infants born before 28 weeks' gestational age were randomized into two groups as in the SUPPORT trial (low 85-89% and high 91-95%). The main outcome for this study was death and disability at the age of two years. The Australian trial resulted in mortality or disability rates of 45% in the lower saturation target group compared to a rate of 39.8% in the higher target group. While the United Kingdom trial resulted in death or disability rates of 50.5% in the lower saturation group compared to 45.9% in the higher target group [94].

A meta-analysis was conducted for targeted oxygen saturations in this population (this included the five randomized control trials Vauche 2012, Schmidt 2013, BOOST NZ 2014, BOOST-II UK 2016, and BOOST-II Australia 2016) [95]. This analysis aimed to find the effects of targeting lower (85-89%) versus higher (91-95%) oxygen saturation on death and major neonatal morbidities in extreme preterm infants. Targeting a lower oxygen saturation range significantly increased the mortality rate at corrected age of 18 to 24 months, mortality rate prior to hospital discharge and significantly decreased the rate of oxygen supplementation at 36 weeks PMA, meeting the BPD criteria [95] .

1.6.2.3. Targeting appropriate SpO_2 range

Pulse oximetry is the most common non-invasive device used in critical care settings for continuous monitoring of SpO_2. Preterm infants usually require respiratory support to prevent hypoxaemic episodes. The hypoxaemic episode is detected by pulse oximetry and the HCPs respond by increasing the fraction of inspired oxygen (FiO_2) temporarily in order to correct the hypoxaemia. Titrating the FiO_2 to target optimal saturation can decrease complications linked with hyperoxia, such as BPD [96]. The manual titration of FiO_2 is the main method of maintaining SpO_2 in the target range. However, it is very difficult and challenging for NICU nurses to be compliant with these clinical guidelines [97].

Several studies have investigated the percentage of monitored time that SpO_2 values were in or outside the target range (Table 1.6). In these studies, the SpO_2 was out of the target range between 32% and 67% of the time for preterm infants receiving oxygen therapy. Whilst it is clear that targeting optimal SpO_2 range in high-risk preterm infants reduces mortality and morbidity, in reality there are challenges achieving this and understanding these could bring about improvements.

Table 1.6 Summary of studies investigating compliance of targeting SpO_2 and reported time outside of the intended target range.

Author	Year	Design	Objective	Target range	Time out of target range
Hagadorn *et al* [98]	2006	Prospective multicentre study	Determine the compliance of targeting SpO_2	Centre specific	52%
Lim *et al* [99]	2014	Observational study	Determine the compliance of SpO_2 targeting while infant on CPAP	88-92%	67%
Van der Eijk *et al* [100]	2012	Observational study	Quantify manual adjustment of FiO_2 for ELBW infants	88-94%	46%
Claure *et al* [101]	2009	Pilot clinical trial	Assess efficacy of automated system in targeting SpO_2	88-95%	58% (manual) 42% (automated system)
Arawiran *et al* [102]	2014	Observational study	Assess the impact of educational intervention	85-92%	56% before intervention, 60% after
Gajdok *et al* [103]	2018	Randomised crossover study	Assess efficacy of new automated system	88-96%	32% (manual) 22% (automated systems)
Reynolds *et al* [104]	2018	Randomised crossover study	Assess efficacy of new automated system	90-95%	51% (manual) 20% (automated system)

1.6.2.4. Clinical challenges affecting oxygen saturation targeting

The two methods to adjust FiO_2, to optimise the percentage of time SpO_2 kept in the appropriate target range, are automated oxygen systems or the manual adjustment of FiO_2. The development of automated oxygen systems mostly built into ventilators could be helpful. These systems detect hypoxaemia or hyperoxaemia and automatically respond by increasing or decreasing FiO_2 to keep

the oxygen saturation in the target range [105].There have been many algorithms used in the automated systems and they remain under continual development [105]. There are a number of drawbacks using an automated system including: high cost, the automated system is often built into a mechanical ventilator, and the response by increase of FiO_2 is not always the most appropriate action to perform such as hypoxaemia caused by hypoventilation. The use of automated FiO_2 systems may reduce the attention of HCPs and delay the recognition of respiratory deterioration. Closed loop systems depend on the accuracy of the FiO_2 analyser and pulse oximeter, but the presence of motion artefact may have an effect on the system's reliability. In addition, few studies demonstrate the impact of using an automated system for improving clinical outcomes such as survival and BPD. Therefore, improving the healthcare teams practice in using manual methods could achieve the goal in targeting SpO_2. The success of manual FiO_2 titration depends on many factors; including alarm limits settings, HCPs knowledge, and clinical stability of preterm infants.

1.6.2.4.1. Alarm limits

The use of alarm limits must be separated from the desired target range. The target range is the clinical goal and limits are utilised to aid in achieving this goal. In clinical settings, alarm limits are ideally set slightly beyond the target range [106]. SpO_2 alarm limits used to warn HCPs to avoid potential harm associated with a SpO_2 outside of the target range.

The AVIOX study examined factors associated with improving SpO_2 targeting for extremely preterm infants [98]. This study found that tight SpO_2 alarm limits were associated with an increased percentage of time in the target range. They defined tight SpO_2 alarm limits to be 1% above the target range for the high limit and not more than 2% below the target range for the lower SpO_2 alarm limit [98]. A recent review evaluated the weakness of SpO_2 targeting clinical trials in preterm infants

defined the appropriate SpO_2 alarm limits to be set no more than 1% or 2% above or below the defined target range [107].

Alarm limits are subject to several limitations that could interfere with targeting SpO_2. The most common problems with alarm limits are fault alarm (e.g., probe or electrode displacement) or alarm fatigue when a caregiver exposed to large number of frequent alarms. This may contribute to a delay with the alarm response time or disregard the alarm altogether [108]. A study conducted to assess the compliance of setting SpO_2 alarm limits correctly in preterm infants found that the upper alarm limit was changed if it became difficult to maintain SpO_2 within the target range. This change occurred due to concerns of hypoxia [108].

Laptook *et al* performed a study to examine the effect of lowering the SpO_2 upper alarm limits from 1% above target range to match the upper target range in very low birth weight infants [109]. They reported that this change resulted in the SpO_2 targeting improving by only 2% [109].

1.6.2.4.2. Personnel factors

Several studies investigated HCPs' compliance in maintaining SpO_2 in the target range. There were several factors thought to play a role in low compliance: lack of awareness of the targeting range, poor knowledge regards the impact of hypoxaemia/hyperoxaemia, and high nurse-patient ratios [99, 109-111]. Education and training could improve the percentage of time SpO_2 kept within the appropriate target range [110, 112].

1.6.3. Detection of cardiorespiratory deterioration in preterm infants

The current method of recognising clinical deterioration depends on physician, nurse, or the parents' experience of detecting worsening clinical condition or changes in clinical measures such as increasing oxygen requirement. The use of early warning scoring systems in both adult and paediatric settings are common and can help identify patients in need of escalation in management. However, these

systems are not yet well developed in neonatal intensive care settings or for preterm infants [113]. Earlier recognition of preterm infant deterioration could reduce the need to escalate respiratory therapy, such as ventilation, and so reduce the risk of lung inflammation and BPD.

1.6.3.1. Early warning systems

Early warning systems, which are also known as physiological track and trigger systems, are used to evaluate physiological parameters for the purpose of early identification of critical illness [114].

Holme *et al* published a retrospective evaluation of a neonatal trigger score (NTS) [115]. This scoring chart used five mandatory items: temperature, heart rate (HR), respiratory rate (RR), respiratory distress, and level of consciousness, in addition to an optional pre-feed blood sugar. Each item was scored between 0-3 giving a total score of 0-15. The higher score conveys a larger deviation from normal with a score of two or more indicating a need for NICU admission. The sensitivity and specificity of NTS was 77% and 97% respectively and was more sensitive than the paediatric early warning scores (PEWS) [115].

PEWS have been developed in predicting patient deterioration [116].This is a score system with three domains involved: behaviour, respiratory, and a cardiovascular and uses a variety of vital signs and symptoms such as HR, RR, body temperature, capillary refill time and administered FiO_2. The PEWS has been found to alert teams early by more than 11 hours compared with routine observation systems. This time period is enough to modify management and prevent deterioration [116].

Roland *et al* developed a newborn early warning system (NEW) [117]. This system was based on risks, including prenatal, perinatal, and postnatal factors. The NEW chart included temperature, HR, RR, apnoea, SpO_2 level, and level of consciousness. All these values classified as green (normal), amber (upper range of normal measurements), or red (abnormal). However, the NEW system was only

able to identify infants at risk in about half of cases who required intervention [117].

Ahmed *et al* developed the Burton newborn observation track and trigger (NOTT) chart that included temperature, RR, and SpO_2. The NOTT chart is used only for infants admitted to postnatal wards with defined risk factors, such as a scalp pH<7.0 or maternal infection. The sensitivity and specificity of the NOTT chart was 96% and 90% respectively for the need of urgent medical assessment and intervention [118].

These warning systems need to be investigated more with prospective studies involving larger sample numbers. Moreover, many factors may influence the effectiveness of these systems such as the inaccuracies of recorded data and scoring, which may impact the main goal of the systems to predict early deterioration. Furthermore, HCPs compliance with the completion of these charts may also impact the effectiveness of the warning systems. Importantly, these systems did not capture continuous physiological data and input measurements were based on episodic data that may not necessarily reflect the real condition of preterm infants.

1.6.3.2. Use of Machine learning systems in the neonatal setting

1.6.3.2.1. Machine learning

Machine learning is defined as the ability of computers to make a successful prediction of outcomes using previous experiences [119]. In clinical science, it is possible to model the association between observant data (input) and related variables (outputs). However, this concept is hard to implement in a real clinical scenario duo to the complex relationship between input and output data. Therefore, machine learning provides methods that build a computational model using a proportion of the data for training and the remaining data to test the model. The process of developing machine-learning model is presented in

Figure 1.5.

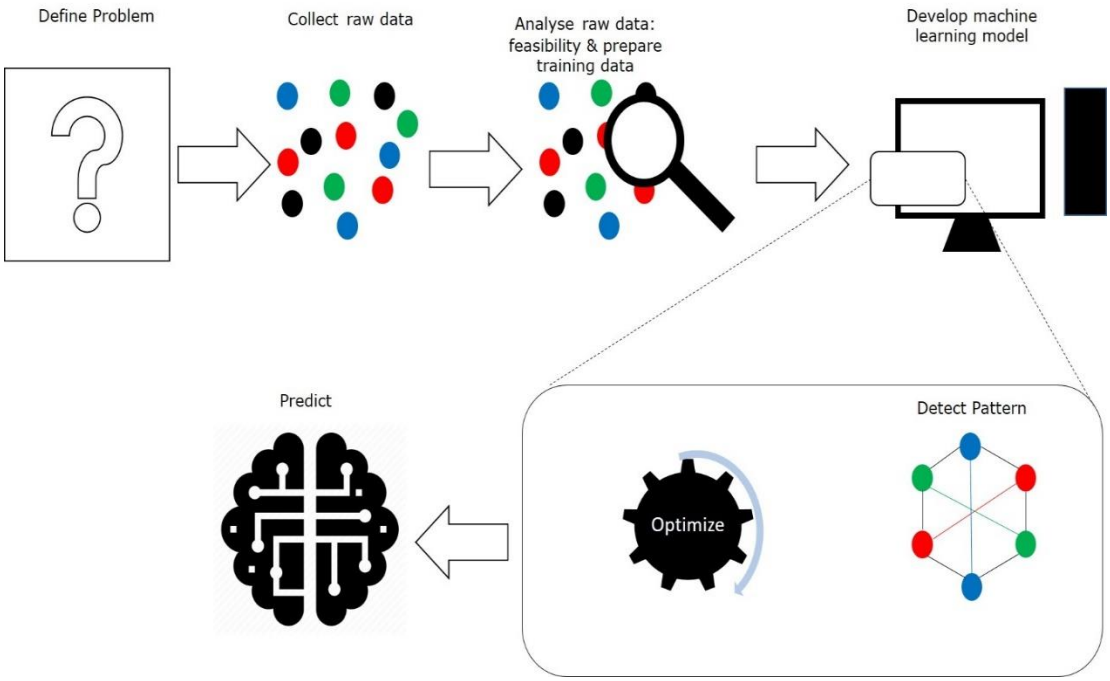


Figure 1.5 Different steps involved in developing a machine-learning model

1.6.3.2.2. Example of intelligent monitoring system in the neonatal setting

There are other novel methods used to identify infants susceptible to clinical deterioration such as HR characteristics monitoring (HeRO) system. HeRO was found to detect infection before the clinical presentation of signs occurred. Reduced HR variability and transient decelerations may present hours to days prior to a diagnosis of neonatal sepsis. Moorman *et al* conducted a randomized control trial of 3003 VLBW infants to investigate the HeRO system [120]. This study measured HR variability and transient decelerations as a potential method to 'warn' the clinical team of possible sepsis earlier than traditional clinical methods. They found that infants who were monitored via HeRO had 2.3 more days alive and off the ventilator compared to infants without a HeRO monitor, reducing the mortality rate from 10.2% to 8.1% although this was not statistically significant. However, a number of negative aspects were identified with this device. The device continuously

displays a risk of sepsis and this was associated with an increase in medical examinations and more days of antibiotics for infants using the monitor. Infants in the HeRO group had 10% more blood cultures taken and 5% more days on antibiotics [121]. In addition, the abnormal HeRO score is not specific to sepsis and presents in other pathological conditions such as respiratory deterioration without ongoing infection [122]. These outcomes highlight the importance of not using single measures as the only factor in clinical decision-making. New algorithms incorporating other vital signs may enhance the diagnostic utility of monitoring and result in further improvement in patient outcomes to overcome these issues.

1.7. Overview of thesis: Main aims and hypothesis

BPD can be a devastating lung disease in preterm infants affecting patients, their families, and wider society. As the literature review has shown, BPD is mainly the result of prematurity, RDS, high oxygen exposure, and positive pressure ventilation. Therefore, any advances in improved monitoring, avoidance of ventilation and reducing oxygen toxicity will help reduce BPD. The present thesis aimed to describe current BPD rates in the England and Wales, understand current respiratory management in NICUs, and explore other potential risk factors and barriers to applying evidence known to reduce BPD.

The broad aim of this thesis is to understand the current challenges facing the respiratory care of preterm infants and implement new interventions to overcome these challenges and improve respiratory management.

The following chapters were designed to assess these aims. Figure 1.6 presents the structure of the thesis.

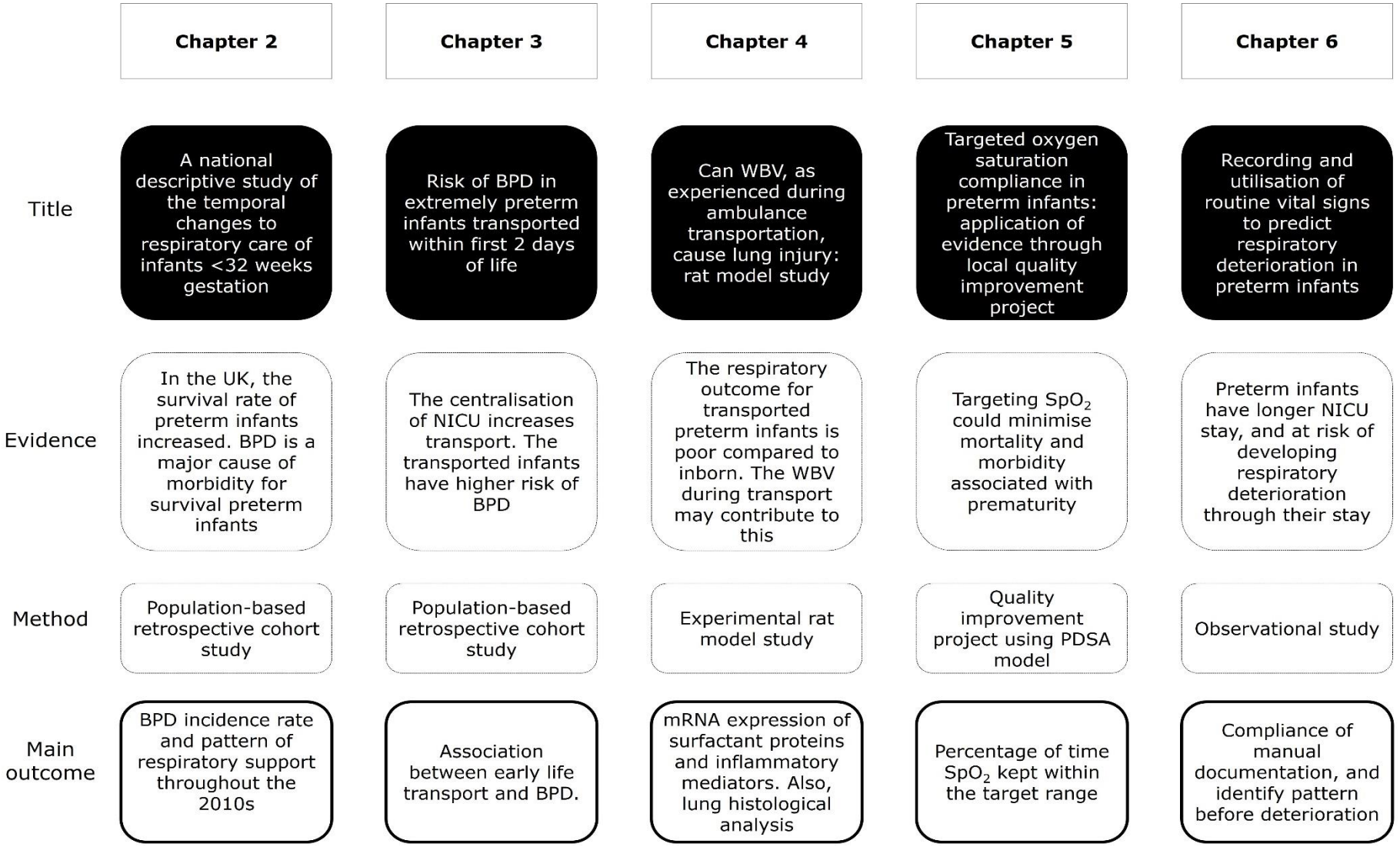


Figure 1.6 Thesis flowchart diagram

Chapter 2. A national descriptive study of the temporal changes to respiratory care of infants <32 weeks gestation

2.1. Overview

Despite improvements in the survival of infants born <32 weeks' gestation over the last two decades, more of these survivors have a diagnosis of BPD [11, 60, 123]. BPD remains one of the most common conditions affecting preterm infants and links with long-term impact on child health, the family, healthcare systems, and the economy [124]. Children and adults diagnosed with BPD in early life are prone to long-term respiratory and neurodevelopmental morbidity [61-63, 125].

The EPICure study reported that an extremely preterm infant in the 11th year of life has significantly higher annual healthcare, social care and educational cost differences of approximately £2,500 per annum compared to term-matched child, the cost was expressed for the financial year 2006-2007 [126]. A recent financial model from Canada has been developed to estimate the lifetime economic burden of an extremely preterm infant diagnosed with BPD [127]. The model reported that an infant with BPD during their lifetime incurs over 700,000 Canadian dollars (approximately £400,000) from healthcare costs.

Since 2007, the NNAP, set up by UK Department of Health and carried out by Royal College of Paediatrics and Child Health, has monitored the quality of care provided by NICUs in England, Scotland, and Wales [35]. The first time NNAP reported the rate of BPD was in the 2016 report, which included rolling three year data (i.e. 2013 to 2015) for preterm infants below 32 weeks of gestational age (Table 2.1).

Table 2.1 Incidence of bronchopulmonary dysplasia (BPD) reported by UK National Neonatal Audit Program (NNAP) in infants <32 weeks gestation.

NNAP annual report	Years covered by data	BPD incidence
2016	2013-2015	30%
2017	2014-2016	31%
2018	2015-2017	31%
2019	2016-2018	31.5%

NNAP reports aim only to assess the quality of services provided by UK neonatal units, so they do not take into account the systematic research approach of reporting the details of BPD in more depth. Infant characteristics or respiratory management are not reported with BPD rates. Moreover, all NNAP reports before 2018 used the old BPD definition that takes into account oxygen dependency at 28 days of age, a definition whose validity has been questioned as discussed in the introduction chapter section 1.5.1.2. [128]

There are no recent studies describing the incidence of BPD and the pattern of respiratory support over the last ten years for infants born <32 weeks gestation in England and Wales. Therefore, the current study aimed to describe the BPD incidence and to explore the pattern of respiratory support modalities for preterm infants below 32 weeks gestational age in England and Wales over the period from 2010 to 2017, by making a large-scale comparison between two equal time epochs (i.e. 2010 to 2013 vs 2014 to 2017). Of note, the NNAP reports cover English, Scottish, and Welsh data. The Scotland data is not included because the release of data required additional ethical and governance processes. However, the sum of these preterm infants <32 weeks gestation born during the period from 2010 to 2017 is 3,727 [129], accounting for <5% of the total dataset used.

2.2. Study aims and hypothesis

2.2.1. Study aims

To describe the difference in BPD incidence for preterm infants below 32 weeks of gestational age in England and Wales between two epochs (i.e. 2010 to 2013 vs 2014 to 2017). In addition, to describe the pattern of respiratory support modalities for the same population and epochs.

The time epoch was based on the period before and after the publication of the BOOST II trial that defines the appropriate oxygen saturation to be 91-95% [130].

2.2.2. Hypothesis

As survival of preterm infants improves, the incidence rate of BPD among infants below 32 weeks of gestational age in England and Wales continues to increase.

2.3. Methods

2.3.1. Theoretical model of the ecological study

Ecological study is an epidemiological observational study in which the unit of the analysis is the population, rather than individual [131, 132]. Ecological studies are commonly used to assess the prevalence and incidence of disease; they monitor population health and direct future public health strategies [132]. There are several types of ecological study including longitudinal studies where a population is examined in order to assess the change of disease incidence over time [132].

Ecological studies usually use routinely collected data, making them inexpensive and easy to conduct, but they have a disadvantage related to the differences between areas in recording disease incidence known as "ecological fallacy" [132]. The ecological fallacy is a term that represents the biases occurred due to the difference between variables at the aggregate level and individual level [131]. Another way of thinking about this is that a percentage of nosocomial infection is high in the hospitals for country X, but that does not necessarily mean every hospital has poor performance, there will be hospitals with lower nosocomial infection rate despite the poor performance of other hospitals.

Ecological studies are the optimal first step in generating hypotheses or directing further investigation for a causal relationship between exposures and outcomes [133].

2.3.2. Study design and setting

This is a population-based retrospective study of all preterm infants born before 32 weeks' gestation and admitted to neonatal units in England and Wales from January 1st 2010 to December 31st 2017.

2.3.3. Data source

Case data were extracted from the National Neonatal Research Database (NNRD). NNRD is a large clinical dataset that contains anonymised data of preterm infants extracted from 185 neonatal units within England and Wales utilising BadgerNet.

This national database provides an electronic record of routinely collected infant data for admissions to neonatal units throughout the UK [134]. The NNRD holds approximately 450 predefined data items including descriptive (e.g. gender), daily (e.g. mode of daily respiratory support), and episodic (e.g. inter-hospital transfer)[134].

There were 185 neonatal units who contributed to the NNRD with over 90% of English units between 2010 and 2012 and 100% of all English and Welsh units by end of 2012. More details regarding NNRD data items can be found on the database website [134].NNRD data validity has been reviewed and found that the quality of these data are high [135]. All episodic data were merged to create single record for each preterm infant.

2.3.4. Study Population

This study included all preterm infants admitted for neonatal care with a gestational age <32 weeks in England and Wales. Of note, infants who die in the delivery room may not be registered in the database.

2.3.4.1. The inclusion criteria

- Gestational age < 32 weeks.
- Admitted to a neonatal unit in England and Wales and have data recorded in the NNRD.
- Born between January 1st 2010 to December 31st 2017.

2.3.4.2. The exclusion criteria (

2.3.4.3. Figure 2.1)

- Preterm infants with birthweight Z score less than -4 or more than +4, according to UK WHO growth chart, were excluded to remove the chance of erroneous entries.
- Infants born with a gestational age less than 23 weeks were excluded due to their small numbers, poor survival and respiratory outcomes.
- Infants missing the BPD classification, which is defined below in the variable definition section.

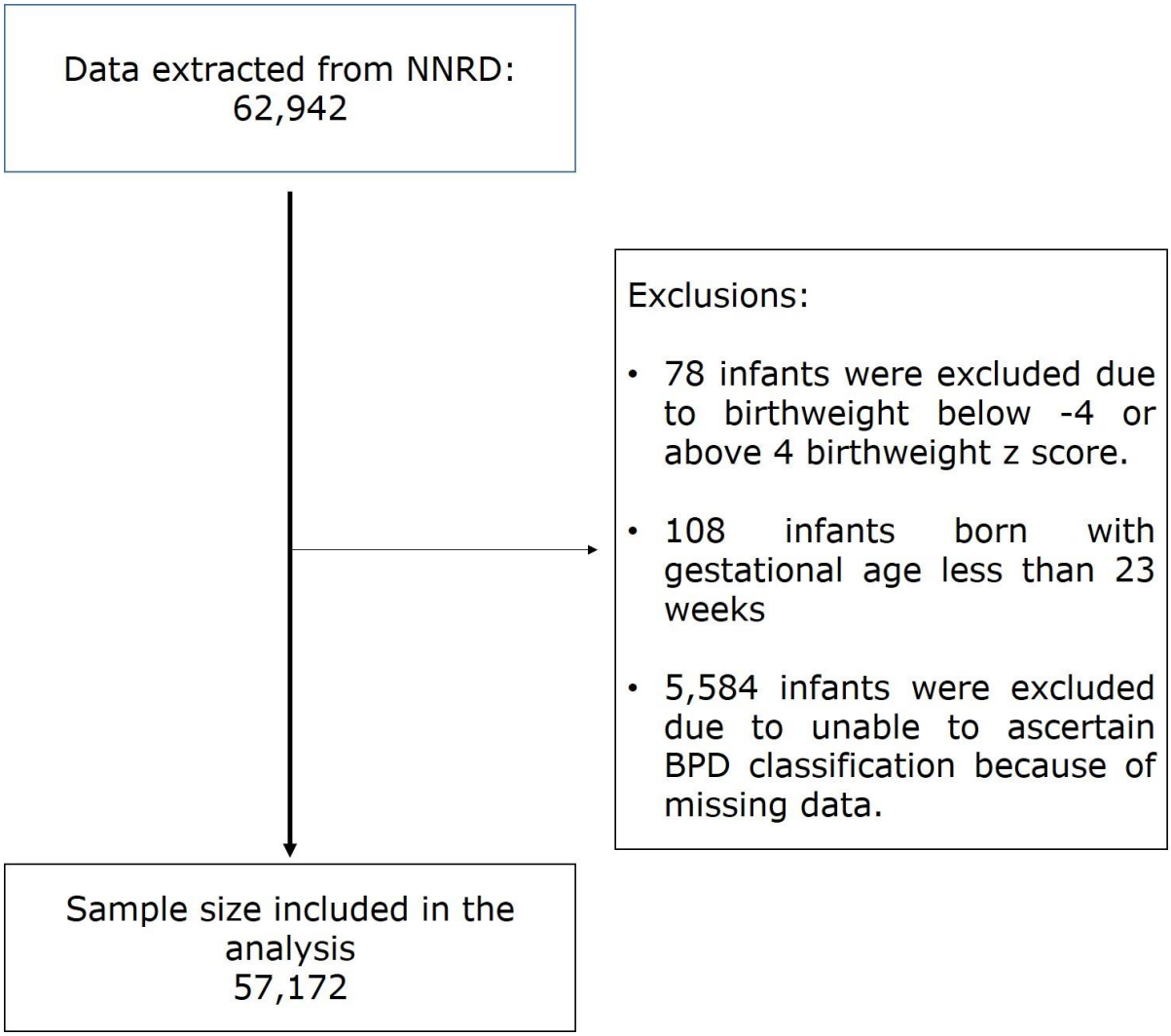


Figure 2.1 Participant flow chart with exclusions and the final sample size

2.3.5. Outcome measures

2.3.5.1. Main outcomes

- To describe the annual trend for mortality of all infants according to birth year.

- To describe the annual trend of BPD incidence for all infants and by gestational subgroups.

- To describe the patterns of use for each respiratory support modality over the years.

- To describe the duration of use for each respiratory support modality over the years.

2.3.5.2. Secondary outcomes

- To determine and compare the incidence of BPD between two epochs (first epoch from 2010 to 2013, and second epoch from 2014 to 2017).
- To describe the respiratory support during the same epochs. This is composed of the number of infants receiving the different methods of respiratory support per birth year and the number of days on each respiratory support modality.
- To describe the incidence of BPD rate according to gestational age subgroups; <28 gestational weeks and 28-31gestational weeks.

2.3.6. Definitions and explanatory of variables

BPD was defined as the dependence on any form of respiratory support or oxygen therapy in surviving infants at 36 weeks of PMA, if infants were discharged before reaching the age of 36 weeks of PMA, the mode of respiratory support used was at the point of discharge [136]. This BPD definition aligns with the recently published evidence [136] and latest NNAP report (i.e. 2018 and 2019 reports) [15, 35]. Respiratory support at 36 weeks PMA was compared with that at 35+6 and 36+1 weeks to verify the reliability of the data at 36 weeks PMA.

Respiratory support data was obtained by using a combination of four different variables in the NNRD database. If there is any discrepancy between the four variables, the highest respiratory mode was used. Infants were considered to be receiving respiratory support (i.e. MV, continuous positive airway pressure (CPAP), HF, and oxygen therapy without any form of respiratory pressure support) if they required at least one day on the stated respiratory modality. The duration for each respiratory support modality indicates the number of days received during the entire neonatal stay. The descending rank of respiratory support was as follows: MV, CPAP or HF, and finally oxygen therapy. If discrepancy showed that infants received both CPAP and HF in one day, they would be excluded from the analysis as it out of the study scope. Infants received both CPAP and HF on the same day

were 8,089 and the median number of days were 1 with interquartile range of (1-2), during the entire study period.

Data on the first admission episode was used for defining gender, birthweight, and gestational age variables. There were less than 1% of infants with a difference in the stated variables between episodes.

The mortality rate was defined as death before discharge from the neonatal unit at any time*;* mortality rate assessment included 5,584 infants that were excluded due to missing BPD classification. Therefore, the total of infants included in the mortality analysis is 62,756.

2.3.7. Ethical approval

Ethical approval for this study was granted by Yorkshire and the Humber – Sheffield research Ethics Committee (reference number 19/YH/0115).

2.3.8. Statistical analysis

All continuous variables were subjected to the assessment of normality distribution using Skewness and Kurtosis test as described by D'Agostino [137]. Continuous variables were described based on the data distribution as a mean ± standard deviation (SD) for normally distributed data or median [interquartile range (IQR)] for non-normally distributed. The statistical comparison for continuous variables was conducted using t-test for normally distributed data or Mann Whitney U test for non-normally distributed data. Categorical data were presented as number (percentage), and the statistical comparison was completed using Chi-Square test. A p-value of <0.05 was considered statistically significant. As this is a large sample size study, a significant p-value is likely to be obtained even when the difference between comparisons is negligible. Therefore, the effect size is reported for significant statistical outcomes during univariate comparison; Phi coefficient test is used to calculate effect size for chi-squared test, Cohen's d for t-test, and the effect size for Mann-Whitney test was calculated using the formula:

$$r = \frac{z}{\sqrt{N}}$$

where z value is the number of standard deviations from the mean value and N is the total number of sample [138]. The interpretation of the effect size is illustrated in Table 2.2, the values of interpretation were based in previous literature [139, 140].

Table 2.2 The interpretation of effect sizes [139, 140].

Relative or association size	**Phi coefficient test value (φ)**	**Cohen's d test value (d)**	**r value**
Small	0.05 – 0.10	≈0.20	≈0.10
Medium	0.10 – 0.15	≈0.50	≈0.30
Large	>0.15	≈0.80	≈0.50

A Cochran Armitage test was used to examine the trends of BPD incidence and mortality rate across the birth years. Trend statistical significance was described with $P_{trend} < 0.05$.

Subgroup analysis by gestational age was conducted to evaluate the trend of respiratory support requirement on infants below 28 weeks and between 28 and 31 gestational age, Jonckheera-Terpstra test was used to define the trend of continuous data (i.e. number of days) across birth year.

Statistical analyses were conducted using STATA SE version 16 software (STATA) and graphical outputs produced using Microsoft Excel version 2016 (Microsoft).

2.4. Results

2.4.1. Mortality rate from 2010 to 2017

62,756 infants were included in this study to assess whether mortality changed over the years from 2010 to 2017. Trend analysis revealed that there was a statistically significant decrease in the mortality rate of infants over the years (P_{trend} < 0.001, Table 2.3).

Table 2.3 Mortality rate according to birth year for preterm infants born with gestational age between 23 and 31 weeks in England and Wales.

Outcome	Birth Year								P value for X^2	P value for trend
	2010 (n=7,498)	2011 (n=7,815)	2012 (n=7,892)	2013 (n=7,853)	2014 (n=7,745)	2015 (n=8,003)	2016 (n=8,037)	2017 (n=7,913)		
Mortality n (%)	744 (9.9)	798 (10.2)	781 (9.9)	708 (9.0)	658 (8.5)	661 (8.3)	646 (8.0)	627 (7.9)	<0.001	<0.001

n: number; X^2: chi squared test.
Mortality defined as death before discharge from neonatal unit at any time.
P values for X^2 determined the significant association between mortality rate and birth year.
P value for trend determined the trend in the mortality rate across the birth year (ordinal value).

2.4.2. Demographics

57,172 preterm infants were included in the analyses describing BPD incidence and respiratory support patterns. The demographic characteristics of the infants based on the two epochs are shown in Table 2.4.

The prenatal characteristics included the diagnosis of intrauterine growth restriction (IUGR), chorioamnionitis, maternal smoking in pregnancy, a complete course of antenatal steroids, and infants delivered via caesarean section (C-section) showed a significant difference between the two epochs.

The gestational age, gender, and birthweight were not significantly different and were comparable between the two epochs. Other variables related to postnatal characteristics included, Apgar score at 1 and 5 minutes, surfactant therapy, received at least five days of antibiotic treatment, and diagnose of patent ductus arteriosus (PDA) needing surgical intervention showed a significant difference between the two epochs. The characteristic details table including the number of missing data is attached in Appendix 1.

Table 2.4 Comparison of demographic characteristics for enrolled infants between two epochs

Characteristic	**Epoch1 (2010-2013)** (n=28,000)	**Epoch2 (2014-2017)** (n=29,172)	**P value**
Smoking in pregnancy	5,084 (18)	5,115 (17.5)	<0.001[x]
Intrauterine growth restriction	2,337 (8)	2,859 (10)	<0.001[x]
Chorioamnionitis	973 (3)	1,241 (4)	<0.001[x]
Completed antenatal steroid course	18,714 (67)	20,784 (71)	<0.001[x]
Delivery via caesarean section	15,367 (55)	16,662 (57)	<0.001[x]
Gestation in weeks, median [IQR]	29 [27-31]	29 [27-31]	0.33[u]
Male	15,177 (54)	15,791 (54)	0.86[x]
Apgar 1 min, median [IQR]	7 [5-8]	6 [5-8]	<0.001[u]
Apgar 5 min, median [IQR]	9 [7-9]	9 [7-9]	<0.001[u]
Birthweight g, median [IQR]	1230 [955 – 1,510]	1230 [950 – 1,510]	0.14[u]
Received surfactant	17,409 (62)	16,601 (57)	<0.001[x]
≥1 course of 5 days antibiotics treatment	16,436 (59)	16,002 (55)	<0.001[x]
Surgically treated PDA	637 (2)	373(1)	<0.001[x]

Data presented as n(%) unless otherwise stated; n: number; IQR: interquartile range; PDA: patent ductus arteriosus
[x] :chi-squared test; [u]:Mann-Whitney test.

2.4.3. Incidence of BPD from 2010 to 2017

The graphical trend of BPD incidence is shown in Figure 2.2. The BPD incidence for all infants by birth year showed an increasing linear trend from 28% to 33% over the eight years (P_{trend} <0.001), an 18% relative increase. Extremely preterm infants showed a significant increase of 11% (P_{trend} <0.001), and very preterm infants showed a significant increase of 27% (P_{trend} <0.001). The numerical rate of BPD, including the number of infants, is attached in Appendix 2.

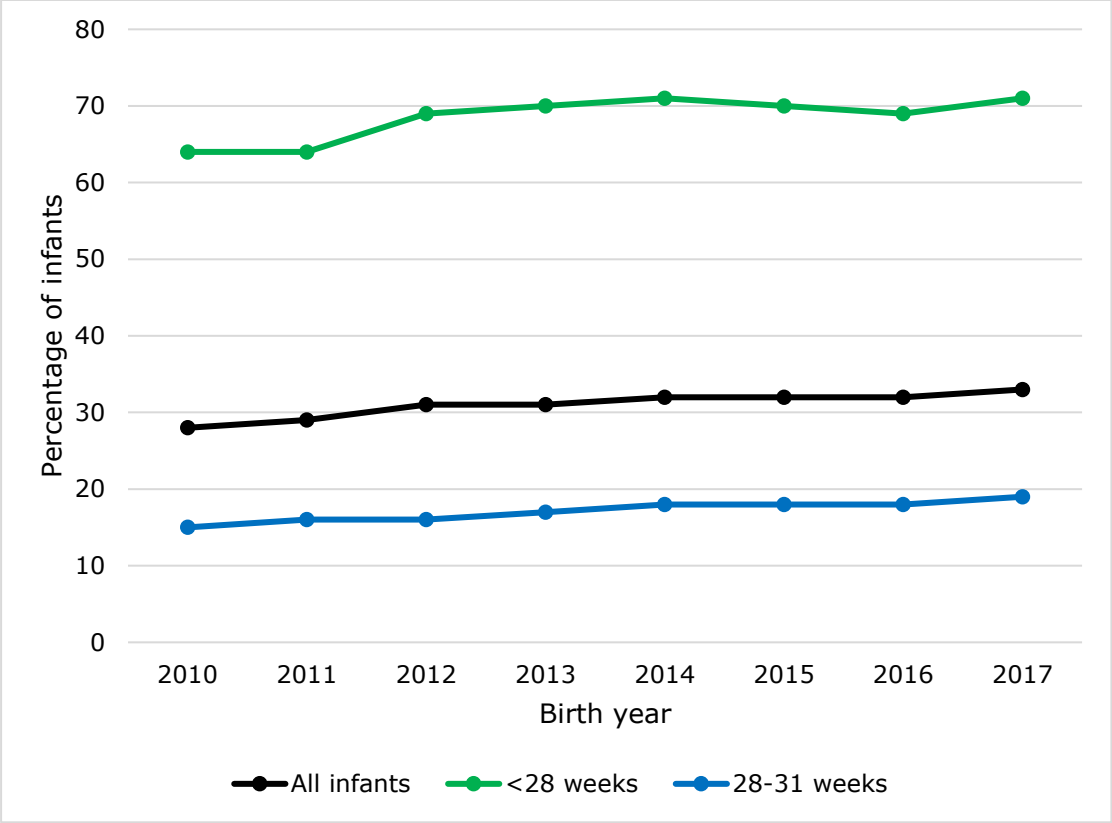


Figure 2.2 Bronchopulmonary dysplasia (BPD) percentage for all preterm infants born between 23 and 32 weeks of gestational age (n=57,172) and by gestational subgroups: <28 weeks of gestational age (n=15,543) and 28 to 31 weeks of gestational age (n=41,629) according to respective birth year.

2.4.4. Change in BPD incidence between epochs

The incidence of BPD significantly increased from 30% (n=8,369) in the first epoch to 32% (n=9,390) in the second epoch ($P<0.001$). The effect size analysis revealed minimal effect of the different epochs on increasing BPD incidence ($\Phi=0.03$). However, a 7% increase in disease incidence could be clinically important.

2.4.5. BPD incidence by gestational age

The graphical output of BPD incidence, according to gestational age, is shown in Figure 2.3. The graph demonstrates BPD is inversely related to the gestational age, as BPD incidence rate decreases with increasing gestational age.

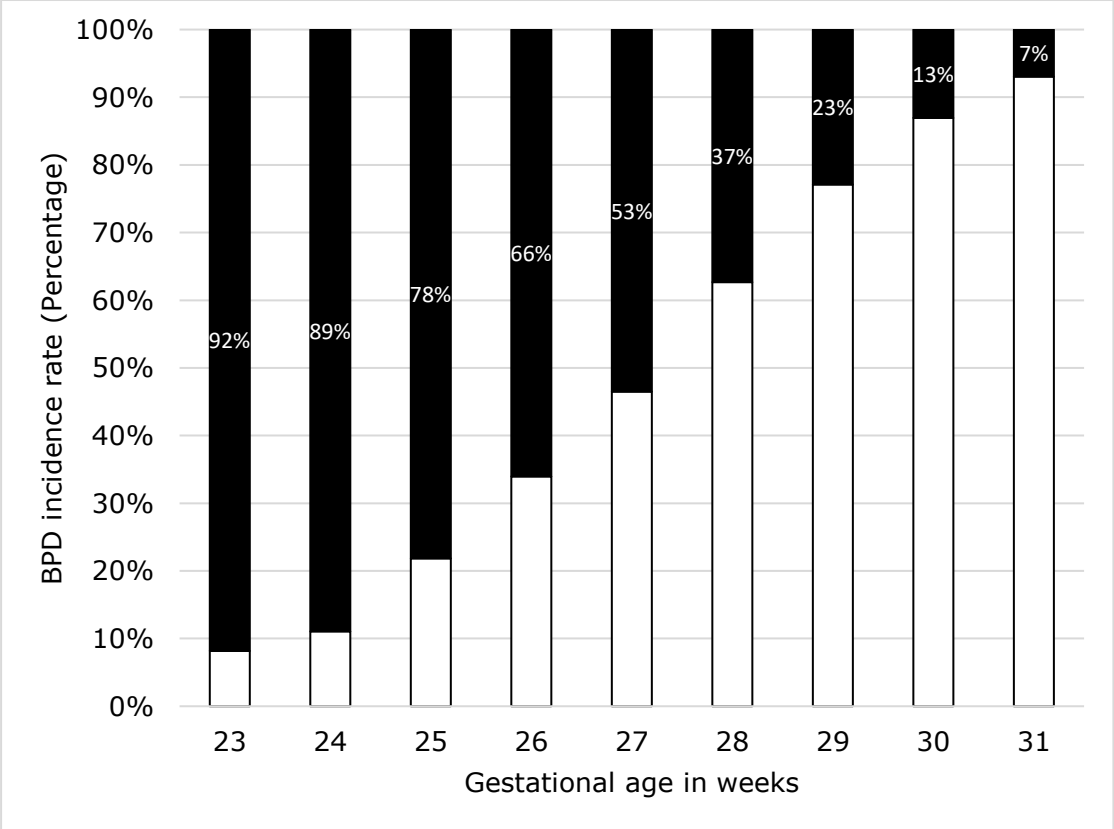


Figure 2.3 Percentage of preterm infants born between 2010 and 2017 (n=57,172) in the respective gestational age category diagnosed with bronchopulmonary dysplasia (BPD). The white bar indicates percentage of no BPD diagnosis and the black bar indicates those with BPD.

2.4.6. Respiratory support patterns 2010 to 2017

2.4.6.1. Percentage of infants using different respiratory support

The annual trends of each respiratory support modality used from 2010 to 2017 in conjunction with BPD annual incidence rate are shown in Figure 2.4. The pattern of using MV, CPAP, and oxygen therapy remain steady over the years. Interestingly, the number of infants using HF increased over the years.

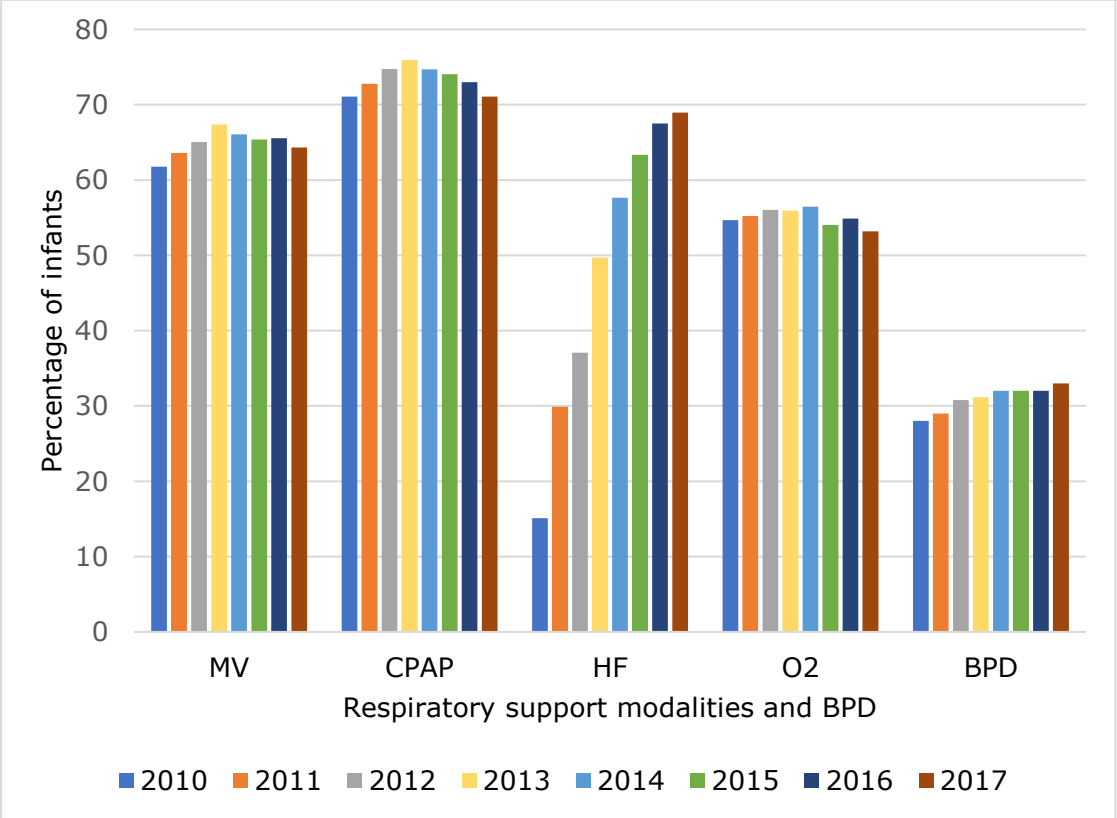


Figure 2.4 Percentage of preterm infants below 32 weeks of gestational age (n=57,172) required at least one day of respective respiratory support modalities (MV=mechanical ventilation, CPAP=continuous positive airway pressure, HF=high flow, O_2=oxygen therapy) during entire stay in conjunction with bronchopulmonary dysplasia (BPD) over birth year from 2010 to 2017 in England and Wales.

2.4.6.2. Duration of respiratory support

Over the study period, the median total number of days that extremely preterm infants (<28 weeks) received respiratory pressure support (i.e. MV, CPAP, and HF) demonstrated an increase from 40 [20-64] days in 2010 to 52 [34-73] days in 2017 ($P_{ascending}$ <0.0001), a 30% relative increase. In addition, the percentage of extremely preterm infants needing any form of pressure respiratory support at 36 weeks of PMA also increased from 32% in 2010 to 44% in 2017, a 37.5% relative increase (Figure 2.5).

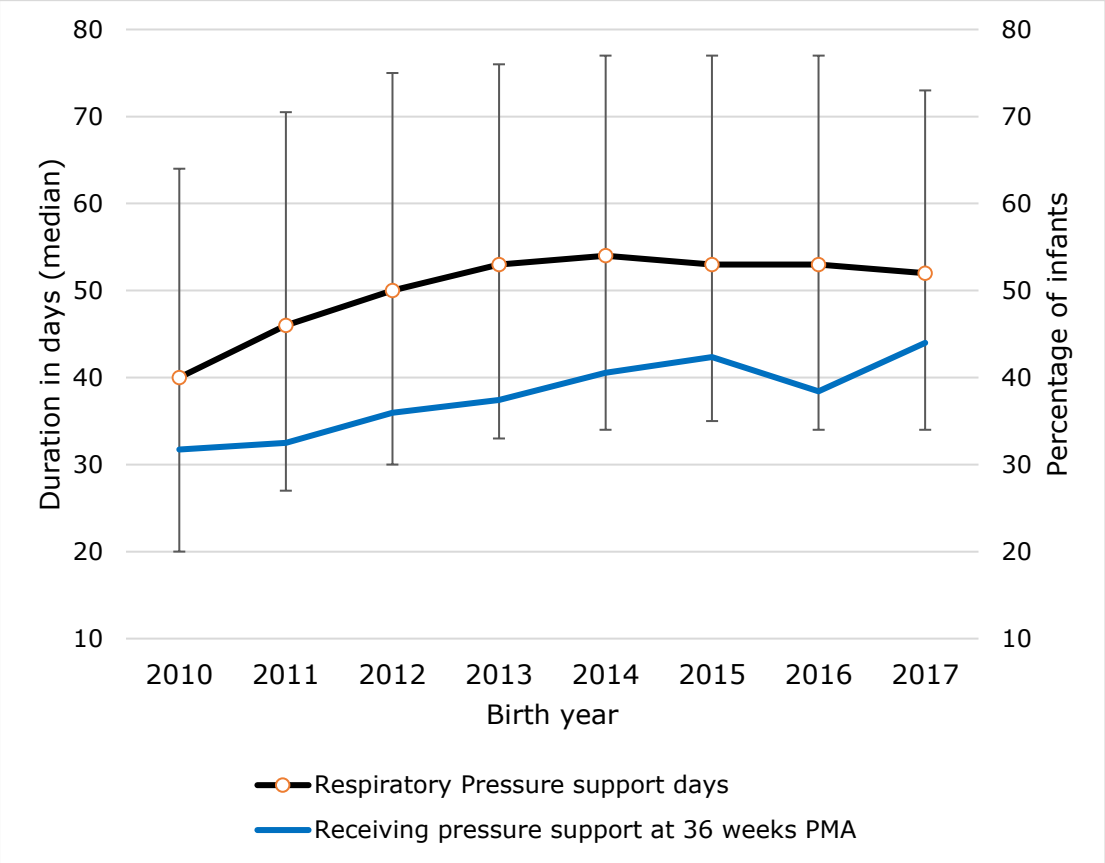


Figure 2.5 Duration in days presented as black line with median (IQR) for infants <28 weeks of gestational age (n=15,543) receiving respiratory pressure support (i.e. mechanical ventilation, continous positive airway pressure, and high flow). The blue line represents the percentage of infants receiving any form of respiratory pressure support at 36 weeks postmenestrual age (PMA).

The total number of days that very preterm infants (28-31 weeks) received respiratory pressure support showed an ascending rate ($p_{ascending}$ <0.0001) that increased from 4 [1-10] days in 2010 to 6 [2-14] days in 2017, a 50% relative increase. In addition, the percentage of very preterm infants who received any form of respiratory pressure support at 36 weeks PMA increased from 5.5% to 8%, a 60% relative increase (Figure 2.6).

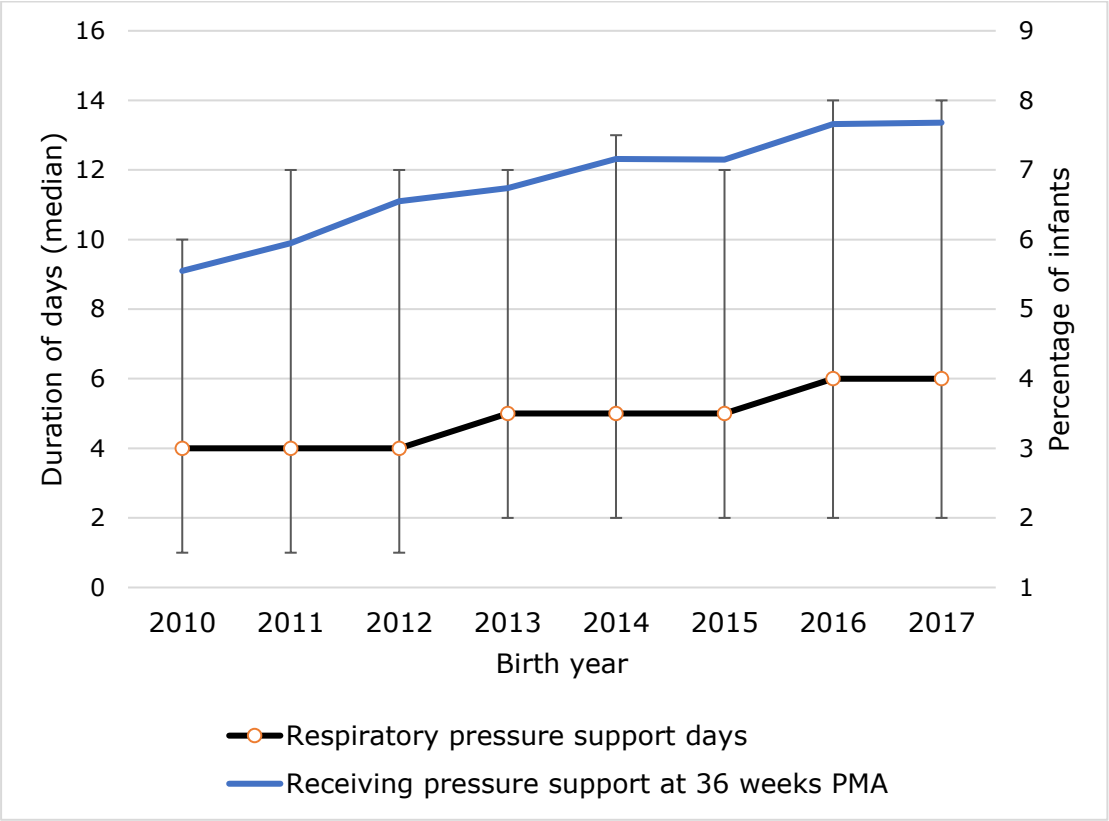


Figure 2.6 Duration in days presented as black line with median (IQR) for infants between 28 and 31 weeks of gestational age (n=41,629) receiving respiratory pressure support (i.e. mechanical ventilation, continuous positive airway pressure, and high flow). The blue line represents the percentage of infants receiving any form of respiratory pressure support at 36 weeks postmenestrual age (PMA).

The duration of MV showed increasing rate over the years for extremely preterm infants ($P_{ascending}$ =0.01). In contrast, very preterm infants showed significant decreasing rate ($P_{descending}$ =0.003).

The duration of oxygen therapy decreased among extremely preterm infants ($P_{descending}$ <0.0001). In contrast, the duration of oxygen showed an increasing rate in very preterm infant ($P_{ascending}$<0.0001).

For extremely preterm infants, the pattern of HF duration in days showed an increasing rate through the study period ($p_{ascending}$<0.0001). In contrast, the pattern of CPAP days significantly decreased throughout the same period ($p_{descending}$ <0.0001) (Figure 2.7).

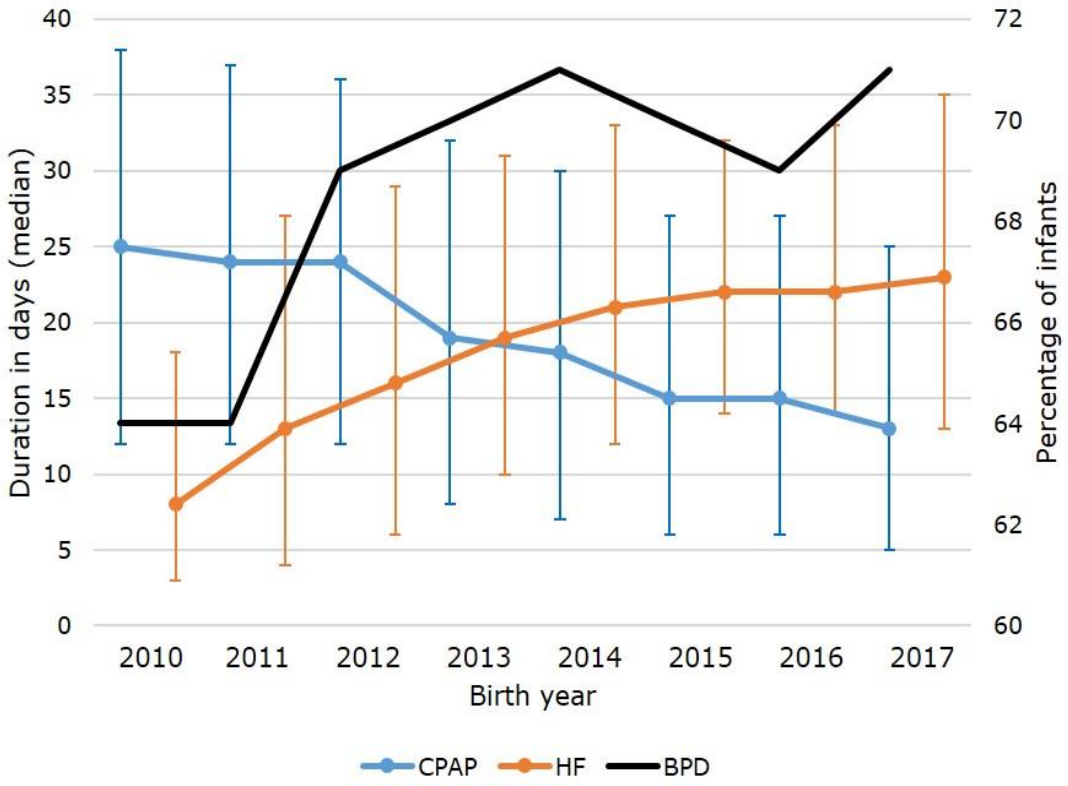


Figure 2.7 Duration in days for continuous positive airway pressure (CPAP, blue line) and high flow (HF, offset orange line) for infants born between 23 and 27 gestational weeks (n=15,543); the duration presented as line graph as median (IQR). The black line represents the percentage of infants diagnosed with bronchopulmonary dysplasia (BPD).

The duration in days for very preterm infants on HF significantly increased ($P_{ascending}$ <0.0001) while CPAP duration significantly decreased ($p_{descending}$ <0.0001) (Figure 2.8).

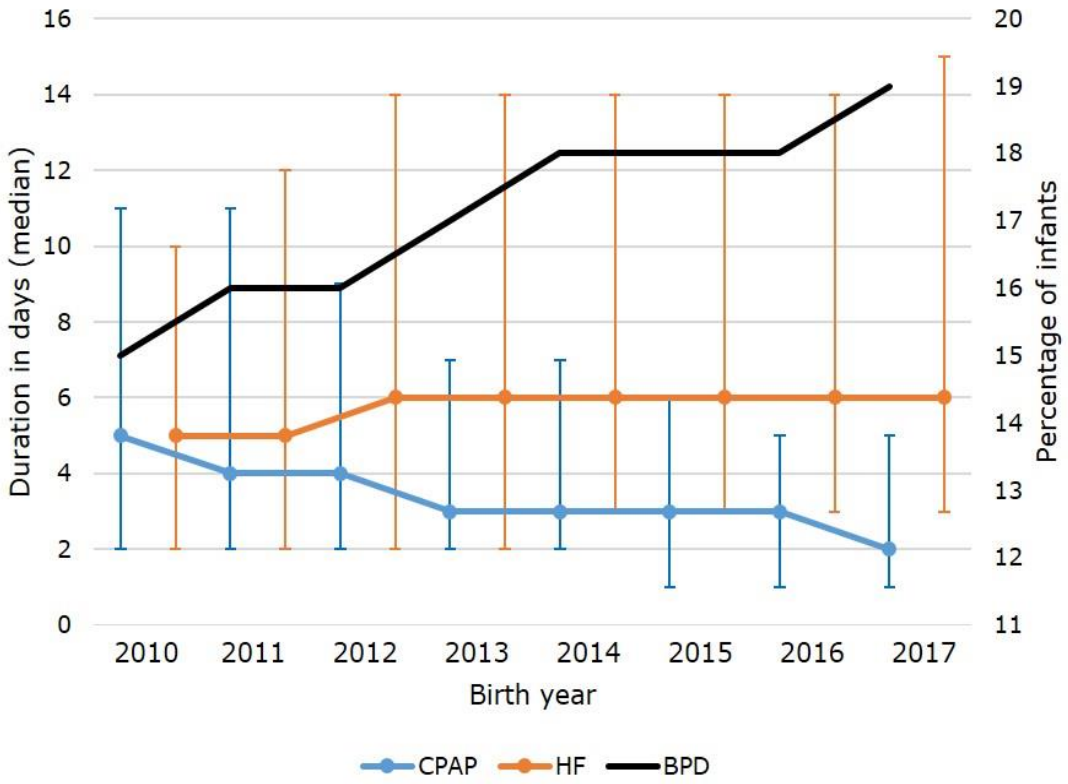


Figure 2.8 Duration in days for continuous positive airway pressure (CPAP, blue line) and high flow (HF, offset orange line) for preterm infants born between 28 and 32 gestational weeks (n=41,629); the duration presented as line graph with median (IQR). The black line represents the percentage of infants diagnosed with bronchopulmonary dysplasia (BPD).

For detailed data, Appendix 3 has the duration in days that infants spent on each respiratory support modality.

2.4.7. Changes in respiratory support between epochs

The comparison of different respiratory support modalities (i.e. MV, CPAP, HF, and oxygen therapy) for managing preterm infants between the first and second epochs are shown in Table 2.5. The use for MV and HF was higher during the second epoch and unchanged for CPAP and oxygen therapy. In the second epoch, the duration of using HF was significantly higher while a significant lower duration of CPAP use was observed.

Table 2.5 Comparison of respiratory support modalities used for infants born <32 weeks gestation according to two epochs

Respiratory support modality	**Epoch 1** (n=28,000)	**Epoch 2** (n=29,172)	**P value**	**Effect size**
Infants needing MV, n (%)	18,057 (64.5)	19,053 (65)	0.04[x]	0.01[Φ]
Duration of MV in days, median (IQR)	4 (2-11)	3 (2-11)	0.24[u]	N/A
Infants needing CPAP, n (%)	20,628 (74)	21,349 (73)	0.19[x]	N/A
Duration of CPAP in days, Median (IQR)	7 (2-20)	4 (2-13)	<0.001[u]	0.1[r]
Infants needing HF, n (%)	9,304 (33)	18,789 (64)	<0.001[x]	0.3[Φ]
Duration of HF in days, Median (IQR)	9 (3-21)	11 (4-24)	<0.001[u]	0.1[r]
Infants on Oxygen therapy, n (%)	15,531 (55.5)	15,938 (55)	0.05[x]	N/a
Duration of oxygen in days, Median (IQR)	14 (4-28)	14 (5-26)	0.05[u]	N/A

n: number; MV: mechanical ventilation; CPAP: continuous positive airway pressure; HF: high flow; IQR: interquartile range
[x] Chi-squared test; u Mann-Whitney test; [Φ] phi coefficient value for effect size;
[r] coefficient for effect size; N/A: not applicable

2.5. Discussion

The present study set out with the main aims of describing the incidence of BPD and the pattern of respiratory support for preterm infants born below 32 weeks of gestation in England and Wales. As more infants survive, the percentage change in BPD incidence has significantly increased from the first epoch to second epoch. The assessment of the change in respiratory support management showed that there was more use of MV and HF in the second epoch, and the duration of HF was also significantly higher in the second epoch. By contrast, the duration of CPAP was significantly lower in the second epoch.

2.5.1. Change in mortality rate

The mortality rate has significantly reduced over the years whilst BPD has increased. This finding supports the association between the improvement in survival for preterm infants and increasing BPD as reported before [36, 141, 142].

Improvements in perinatal and neonatal medicine has resulted in a reduction in the mortality rate for vulnerable preterm infants. The present study identified a significant increase in mothers receiving a complete course of antenatal steroids which may have contributed to the reduction in mortality rate [143]. In addition, it is interesting to note that the rate of PDA requiring surgical intervention was significantly lower in the second epoch. This finding is consistent with the prevailing opinion that failure of spontaneous PDA closure contributed to increasing mortality among preterm infants [144]. However, there is a big debate in the literature regarding the association between PDA surgical closure and improving mortality rate [145]. Therefore, it is hard to relate the reduction in mortality to the reduction in surgical treated PDA and further investigation is recommended.

2.5.2. Change in the BPD incidence

The present study revealed a relative increase of 7% in BPD incidence between the two epochs, with the pattern over the years a significant linear trend increasing by 18% from 2010 to 2017. The increase in BPD matches the NNAP reports [146],

which showed an increase over the previous years in the UK. In this study, the rate of BPD among extremely preterm infants ranged between 64% and 71%, and this result is at the higher end of the latest review that describes the global BPD incidence rate to be between 17% to 75% among extremely preterm infants [45]. The finding that BPD is associated with gestational age supports previous findings that the incidence of BPD is inversely related to the gestational age [36, 147]. Extremely preterm infants, especially below 26 weeks of gestational age, are born with structural and physiological immaturity of the lung, putting them at high risk of BPD development [147, 148].

The antenatal, postnatal, and respiratory management factors will be discussed to understand the potential causes of the high BPD rate in England and Wales that observed in the current study.

There is growing evidence of antenatal risk factors for BPD; these risk factors include IUGR, chorioamnionitis, and delivery via C-section [149]. IUGR is known to impair lung vascular architecture in the fetal lung, and this might contribute to the increased BPD among these infants [150]. Preterm infants exposed to chorioamnionitis are at high risk of developing BPD [151]. Infant delivered via C-section are at higher risk of developing significantly poor respiratory outcomes includes respiratory distress during earlier life and asthma in childhood [152, 153]. These antenatal factors were significantly higher during the second epoch in the present study, potentially contributing to the observed increase in BPD. Equally, the rate of maternal smoking reduced in the second epoch. Maternal smoking has been found to alter in-utero lung development and is strongly associated with the development of BPD among preterm infants [154].

Antenatal steroids are known to reduce mortality and the incidence of neonatal RDS, as it known to accelerate lung development and enhance the pulmonary surfactant system [155]. However, the evidence suggests that it is not effective in reducing BPD rate among preterm infants [143, 156]. It is therefore likely that the

role of antenatal steroids in improving survival rate is offset by the increase of BPD rate as observed in the current study.

The postnatal baseline characteristics revealed that the Apgar score at 1 minute was significantly lower during the second epoch, but only by one point. This finding seems to be consistent with previous research which found that for every point subtracted on the 1 minute Apgar score, the risk of BPD increased among preterm infants [157]. Despite the significant difference in Apgar 5 minute score between the two epochs, the medians were identical in both epochs and this is probably of little clinical significance.

Another important postnatal factor that may contribute to the increase in BPD during the second epoch is the reduced administration of surfactant therapy. Surfactant therapy could reduce BPD incidence by reducing the duration needed for MV and oxygen therapy, both major risk factors for BPD [158]. Recently, there has been a move to reduce the use of intubation to deliver surfactant therapy and replace it with less invasive surfactant administration technique [159], this movement should reduce need for MV and therefore BPD rate. However, my data show that BPD is increasing and the duration of MV also increased for extremely preterm infants, whilst decreasing for very preterm infants. This discrepancy could be explained by that very preterm infants are less likely to receive prophylactic surfactant therapy which could justify the higher need of MV among extremely preterm infants.

Antibiotic treatment and diagnosis of a significant PDA, that requires surgical intervention, are factors found to be associated with increasing BPD incidence, as previously described [160, 161]. However, the findings of the baseline comparison in the current study showed a significant reduction of antibiotic use and PDA during the second epoch, suggesting the increased in BPD may not be directly related to these two factors in this study.

One factor that could significantly increase the BPD incidence is the increase in the number of infants requiring MV during the second epoch [147]. Despite that the

effect size analysis revealed a negligible effect of the second epoch on increasing use of MV and a non-significant difference between epochs in the duration of MV, the annual trend of MV days among extremely preterm infants has increased. This finding could help explain some of the increase in BPD rate observed in extremely preterm infants and it is consistent with a previous study reporting MV duration is strong predictor of BPD especially among extremely low birth weight infants [57]. In contrast, the trend of MV among very preterm infant showed a decreasing pattern, and this finding could not be related to the increase incidence of BPD among very preterm although both rates of prolonged MV and BPD were much lower in this subgroup. However, other respiratory management patterns for very preterm infants could contribute to the development of BPD including the increased use of oxygen therapy. It is well described in the literature that a higher exposure to oxygen therapy in early life is strong predictor for BPD development [162].

The most important finding was the use of HF significantly increased with the rising number of infants and duration in days required. It is plausible that among extremely preterm infants the increase in the duration of HF might be linked with the increase in BPD. Previous literature reported that extremely low birth weight and infants below 30 weeks gestational age managed with HF were at higher risk of BPD development [163, 164]. In contrast, a previous Cochrane review and meta-analysis concluded that use of HF has a similar rate of efficacy to other non-invasive respiratory support in preventing the development of BPD [165, 166]. However, this efficacy was only determined in moderate to late very preterm infants' ≥28 weeks of gestational age. One of the mechanisms of HF is to generate and provide a positive distending pressure [167], and this pressure is varied based on several factors such as the presence of a leak. The unregulated positive pressure might cause lung injury due to either overexpansion or atelectasis, and therefore development of BPD could occur.

In contrast to HF, there was a reduction in the duration of CPAP over the years. CPAP has been described as a protective approach against the incidence of BPD

[168]. Although, it is difficult to relate the increased incidence of BPD to the decreased duration of CPAP. However, it appears in the present study that HF is more likely to be used as a substitute to CPAP. Moreover, the overall need of any form of respiratory pressure support increased through the study years. Considering that the CPAP use, which is protective respiratory support against BPD development [168], were reduced while other forms i.e. MV and HF, implicated in BPD development [147, 163], both increased through the years. Therefore, these three considerations could be related to the observed increase in BPD rate.

2.5.3. Changing respiratory management

The results of this study highlight that among the four respiratory support modalities assessed in this study, only HF and CPAP had a significant change in their duration. The number of infants receiving HF support, and its duration for all infants and gestational subgroups, increased over the years. These findings support the published literature concluding that HF is gaining in popularity and becoming a well-established respiratory support method in the UK and around the world [169, 170].

The duration of HF has gradually increased while the duration of CPAP decreased, especially among extremely preterm infants. This finding could indicate a switching from CPAP to HF in neonatal settings. The rapid implementation of HF in NICUs might be due to several advantages over CPAP, such as reduced nasal trauma, infant comfort, and ease of use while having similar efficacy to non-invasive respiratory support including CPAP [165, 171].

The use of MV has increased over the study period among extremely preterm infants. This finding may be explained by the fact that more infants are admitted to neonatal units and more are surviving so needing MV to manage respiratory distress associated with extreme prematurity. In contrast, the trend of MV days for very preterm infants showed decreasing pattern. This may be related to changing practice in using MV for very preterm infants and it is preferred to use non-invasive

pressure support or oxygen therapy (i.e. nasal cannula and head box) at the beginning for such patients., The median MV duration among very preterm infants is two days throughout the years, however, the significant difference is contributed to the difference in rank sums and sample numbers, which could give same medians but different data spread.

2.6. Study strengths and limitations

2.6.1. Study strengths

The main strength of this study is the large sample size of infants born <32 weeks gestation that represents almost all neonatal admissions in England and Wales during the period from 2010 to 2017. To the best of my knowledge, this is the largest cohort study reporting the annual incidence of BPD and describing the contemporary patterns of respiratory support across a whole healthcare system. The completeness and quality of data held in NNRD are high and have been previously validated [172]. In addition, the nature of using multicentre database minimises the impact of local practices on the outcomes.

This study provides data for policymakers to plan future resources and associated costs for the management of preterm infants during birth, into childhood and adulthood. It also provides researchers with a number of interesting new hypothesis relating to the changing pattern of respiratory management and outcomes in the NHS.

2.6.2. Study limitations

The study has several limitations. During the period from 2010 to 2012, only 90 % of neonatal units' data in England and Wales are included in NNRD database. However, it is unlikely to affect the findings of this study as the percentages were used in reporting the results, not the exact numbers, and it is unlikely that the remaining 10% would vary significantly from the majority. The retrospective nature of this study leads to a loss of approximately 9% of total preterm infants due to the incomplete data to classify BPD. The study was also unable to look at regional

or unit differences, which are known to exist [146]. The baseline characteristics reported in this study aimed to compare BPD incidence rate and not mortality rate. There were 5,584 infants included in the mortality analysis and not included in the baseline characteristics because of missing data and unable to ascertain BPD classification. However, this was equivalent to 9% of total number of infants included and unlikely to shift the finding significantly. Incorrect data entry could not be identified, as this is routinely recorded information. However, the large number of infants studied could smooth out minor irregularities or errors in data input. In addition, the exact amount of oxygen was not reported by the database therefore the impact of oxygen while on respiratory pressure support could not be investigated.

Finally, the causal relationship for multiple factors and BPD incidence could not be investigated using the statistical modelling. Therefore, this limitation must be considered in the context of designing further studies investigating BPD using the NNRD.

2.7. Conclusions

Mortality for infants born <32 weeks gestation is improving for those born in England and Wales from 2010 to 2017. In parallel, the rate of BPD has increased for the same population. The use of HF has rapidly increased during the study period, despite evidence to support any benefits over CPAP, which has reduced in the use during the same period. The longer duration on pressure support ventilation may be one of the additional contributing factors to the rising BPD rate.

2.8. Future works

Despite improvements and advances in NICU management, the rate of BPD continues to increase. Studies exploring the predictors for BPD using relative risk measures are important to understand these increases and identify modifiable factors to reduce the rates.

The differences in the clinical and respiratory management practices among NICUs were not explored in this study. Therefore, it would be worthwhile exploring and comparing the differences in such practices in units with a higher and lower incidence of BPD.

There is scope for further research in determining the correlation between the duration of HF and the BPD rate, especially among extremely preterm infants. This finding could contribute to the growing literature of the safety and the efficacy of using HF among preterm infants.

Chapter 3. Risk of bronchopulmonary dysplasia in extremely preterm infants transported within the first 48 hours of life

3.1. Overview

The centralisation of neonatal intensive care in the UK has reduced the mortality rate of high-risk newborn infants [4]. Extremely preterm infants, born at gestational age below 28 weeks, have an increased risk of developing BPD [45]. The pathophysiology of BPD is a multifactorial process in which different antenatal and postnatal factors could aggravate the normal development of the lung; prematurity is the key factor associated with BPD incidence [36].

The EPICure 2 study reported that extremely preterm infants who were transported in the first day of life, only 7% survived without development of major morbidity [4]. A more recent study compared the outcome between inborn and outborn infants in the United States, and reported that the BPD rate was increased in transported preterm infants [69]. This study assessed the outcome for preterm infants below 30 weeks of gestational age and only included infants transferred on the same day of birth. There are no contemporary data published to assess the risk of BPD incidence after early life transport for extremely preterm infants in the UK.

Understanding the association between BPD and early life transportation among extremely preterm infants could provide useful data for future studies to optimise the management and improve the respiratory outcome of these high-risk infants.

3.2. Study aims and hypothesis

3.2.1. Aims

3.2.1.1. Primary aim

To evaluate the association between early transport, within the first 48 hours of life, and BPD incidence in extremely preterm infants born <28 weeks of gestational age.

3.2.1.2. Secondary aims

1) To identify and compare the mortality rate among extremely preterm infants according to transfer status within first 48 hours of life.

2) To evaluate the association between early neonatal transport and composite outcome of BPD or death in extremely preterm infants according to transfer status within first 48 hours of life.

3) To identify and compare the duration of MV before first extubation in extremely preterm infants according to transfer status within first 48 hours of life.

3.2.2. Hypothesis

Early postnatal transport of extremely preterm infants born is associated with the development of BPD and a longer duration on MV before first extubation compared to inborn infants.

3.3. Methods

3.3.1. Study design

A population-based retrospective cohort study conducted to assess the incidence of BPD in extremely preterm infants comparing those transported within the first 48 hours of life and inborn infants in England and Wales.

3.3.2. Theoretical model of retrospective cohort study

A cohort study is the optimal method for determining the incidence of the interest outcome and natural history of it [173]. Cohort study involves using measurements of participants over period of time to determine whether they develop the disease and identify any association between exposure to risk factors and development of disease [174]. Cohort studies help to understand what risk factors increase the chance of developing a disease.

Retrospective cohort study is typically conducted using data that have already been collected to find the association between risk factor and development of disease [175]. The term "retrospective" originated from the fact that the exposure and outcome had already occurred when the study started. Retrospective cohort study is performed by dividing the participants into two groups. One group exposed to defined risk factor and the other did not, and then define the incidence rate of the disease in both groups.

Retrospective cohort studies are helpful in finding the temporal relationship between risk factor and disease. Also, they can examine different outcome variables related to the exposure risk factor such as death [173]. In addition, cohort studies permit the measurement and adjustment of many other risk factors associated with the development of the disease [173]. Retrospective studies are typically less time consuming and inexpensive to perform [174]. Cohort studies are usually large and sometimes involve whole populations. These studies allow conclusions regarding association between risk factor and disease to be drawn [174].

A retrospective cohort study depends largely on the availability of reliable and accurate data records. A major weakness of retrospective studies are the inability to control for all other risk factors that might differ between the exposed and unexposed groups [173]. Risk of bias can occur in any research and cannot be excluded.

3.3.3. Data Source

The source for the data used in this study was the NNRD, detailed in (chapter 2). Briefly, NNRD is a large UK database that contains de-identified data for all infants admitted for neonatal care in England and Wales.

3.3.4. Study population

This study included all extremely preterm infants born below 28 weeks of gestational age and admitted to neonatal units in England and Wales between 1st January 2010 and 31st December 2017.

3.3.4.1. Inclusion criteria

- Infants born at gestational age <28 weeks and admitted for neonatal care.

3.3.4.2. Exclusion criteria

- Extremely preterm infants with birthweight Z score less than -4 or more than +4, according to UK WHO growth chart [176], were excluded to remove the chance of erroneous entries. (Excluded already during data management step).
- Infants born below 23 weeks of gestational age, due to the unavailability of the z score for them to check their birth weight inclusion criteria.
- Infants with missing data making a BPD classification impossible as previously explained in chapter 2.
- Infants missing the transfer status at 48 hours of age.

3.3.5. Group definition

Data were imported for all extremely preterm infants who were either transported or not to any care level within the first 48 hours of life. The current study defined

the transported group as transported within the first 48 hours of life, a priori determined in previous studies [177, 178]. The inborn group comprised infants born in hospital and not transported within first 48 hours of life.

3.3.6. Outcome definitions

The BPD incidence was determined by assessing the respiratory support at 36 weeks of corrected gestational age as described previously in chapter 2.

A sub-groups analysis was performed to assess the difference in the BPD incidence between transported and inborn extremely preterm infants based on two different gestational age subgroups: 23-25, and 26-27 weeks. Sub-group analysis performed to further explore the impact of prematurity on the incidence of BPD after early transport.

Mortality was defined as death before discharge from the neonatal unit. The composite outcome was defined as death any time before neonatal unit discharge or diagnosis with BPD at 36 weeks gestational age. Infants included in the mortality and composite outcome analysis are described in Appendix 4.

In addition, one of the secondary aims was to assess the duration of MV before first extubation for preterm infants according to the transport status. The extubation was defined as infants successfully maintained off MV for at least 48 hours after their first extubation.

3.3.7. Ethical approval

Ethical approval for this study was granted by Yorkshire and the Humber – Sheffield research Ethics Committee (reference number 19/YH/0115).

3.3.8. Statistical analysis

Infants were divided into two groups according to the transport status at the first 48 hours of life: inborn and transported groups.

The comparison of infant characteristics and outcomes according to transport status were conducted using chi-square tests for categorical variables, independent

t-test for normally distributed continuous variables, and Mann-Whitney U test for non-normally distributed continuous variables. Continuous data were subjected to D'Agostino normality test to define the normality.

As this is a large population study, a significant p value is likely to be obtained even when the difference between comparisons is negligible. Therefore, effect size will be reported for outcomes during univariate comparison. The Phi coefficient test will be used to calculate the effect size for chi-squared test, and the effect size for Mann-Whitney test will be calculated using the formula:

$$r = \frac{z}{\sqrt{N}}$$

where z value is the number of standard deviations from the mean value and N is the number of samples [138]. The interpretation of the effect size is illustrated in Table 3.1 [139, 140].

Table 3.1 Interpretation of the effect size results [139, 140].

Relative or association size	Phi coefficient test value (φ)	r value
Small	0.05 – 0.10	≈0.10
Medium	0.10 – 0.15	≈0.30
Large	>0.15	≈0.50

Logistic regression was used to define the association between the main outcome and the exposure i.e. BPD and early transport before any adjustment to the regression model.

The adjusted odds ratio (aOR) for the association between transport status and BPD incidence was calculated using a multivariable logistic regression model to control for important confounding factors. The confounding factors were included in the model for the following three reasons. Gestational age, gender, and birthweight were included in the model as a priori confounders. In addition, any variable with statistical significance in the univariate analysis according to the

transport status (i.e. comparison between transported and inborn characteristics). Variables that did not show statistical significance in the univariate analysis were individually added into the model as confounders if able to change the aOR in either direction by greater than or equal to 10%.

All statistical analyses were conducted using Stata SE (version 16, StataCorp, TX USA). A p value of less than 0.05 defined the statistical significance.

3.4. Results

3.4.1. Composite outcome of death or BPD according to early transport

Out of 19,586 extremely preterm infants included in the analysis of the composite outcome (i.e. death or BPD), 3,875 infants were transported and 15,711 were inborn. The background characteristics of the infants and their respiratory management are attached in Appendices 5-7.

The composite outcome was significantly higher among the transported group at 77% (n=2,994) compared to the inborn group 74% (n=11,613) (P<0.001). The 4% difference could be clinically important but the effect size of transport status on the composite outcome was very small (Φ value=0.03).

The gestational subgroup analysis revealed that infants with gestational age of 23 to 25 weeks had a borderline lower incidence of the composite outcome when transported 87.5% (n=1,754) compared to inborn group 89% (n=5,996) (P=0.04). In contrast, preterm infants with gestational age 26 and 27 weeks had a significantly higher incidence of composite outcome when transported 66% (n=1,240) compared to the inborn group 62.5% (n=5,617) (P<0.01). The infant characteristics and respiratory management for gestational sub groups are attached in Appendices 8-13.

Extremely preterm infants who had transported had a significantly increased risk of death or BPD compared to inborn infants. However, following adjustment for confounders, the association no longer remained significant (Table 3.2).

Multiple logistic regression analysis for 23-25 weeks and 26-27 week gestations found no association between composite outcome and early life transport, following adjustment for key confounders (Table 3.2). Off note, all gestational, infant, and respiratory management items listed in Appendices 5 - 7 were assessed as per the criteria explained in section 3.3.8 to define which confounders should be included in the logistic regression model. The assessment was conducted for each group separately as there is a variation in univariate analysis output among groups.

Table 3.2 Association of transport with composite outcome i.e. death or bronchopulmonary dysplasia (BPD) for all extremely preterm infants born between 23 and 27 weeks of gestational age, and by gestational subgroups.

Cohort	OR (95% CI)	Adjusted OR (95% CI)
All infants (n=19,586)	1.20 (1.10-1.30)	1.02 (0.90-1.16)[a]
Infants between 23 and 25 weeks (n=8,726)	0.85 (0.73-1.0)	0.93 (0.77-1.12)[b]
Infants between 26 and 27 weeks (n=10,860)	1.18 (1.1-1.31)	1.09 (0.94-1.28)[c]

[a] adjusted for gestational age, gender, birth weight, maternal age, chronic hypertension, preeclampsia, maternal diabetes, smoking during pregnancy, complete course of antenatal steroids, chorioamnionitis, intrauterine growth restriction, multiple pregnancy, mode of delivery, Apgar score at 1 minute and 5 minutes, surfactant therapy, ≥5 day conservative treatment for surgical or medical necrotising enterocolitis, ≥5 days of antibiotic treatment, receive at least one day of mechanical ventilation (MV), receive at least one day of continuous positive airway pressure (CPAP), duration of MV, and duration of CPAP therapy.

[b] adjusted for gestational age, gender, birth weight, maternal age, preeclampsia, complete course of antenatal steroids, chorioamnionitis, intrauterine growth restriction, multiple pregnancy, Apgar score at 5 minutes, surfactant therapy, ≥5 days of antibiotic treatment, and at least one day of MV.

[C] adjusted for gestational age, gender, birth weight, maternal age, chronic hypertension, preeclampsia, smoking in pregnancy, complete course of antenatal steroids, chorioamnionitis, intrauterine growth restriction, mode of delivery, Apgar score at 1 and 5 minutes, surfactant therapy, ≥5 days conservative treatment for surgical or medical necrotising enterocolitis, ≥5 days of antibiotic treatment, at least one day of MV, duration of MV, at least one day of high flow, and at least one day of respiratory support via CPAP.

3.4.2. Study population for BPD at 36 weeks

From the total population of 19,611 infants, 15,543 were included in the analyses of BPD at 36 weeks (

Figure **3.1**). Of these, 3,025 (19%) were transported within the first 48 hours of life, and 12,518 (81%) were inborn.

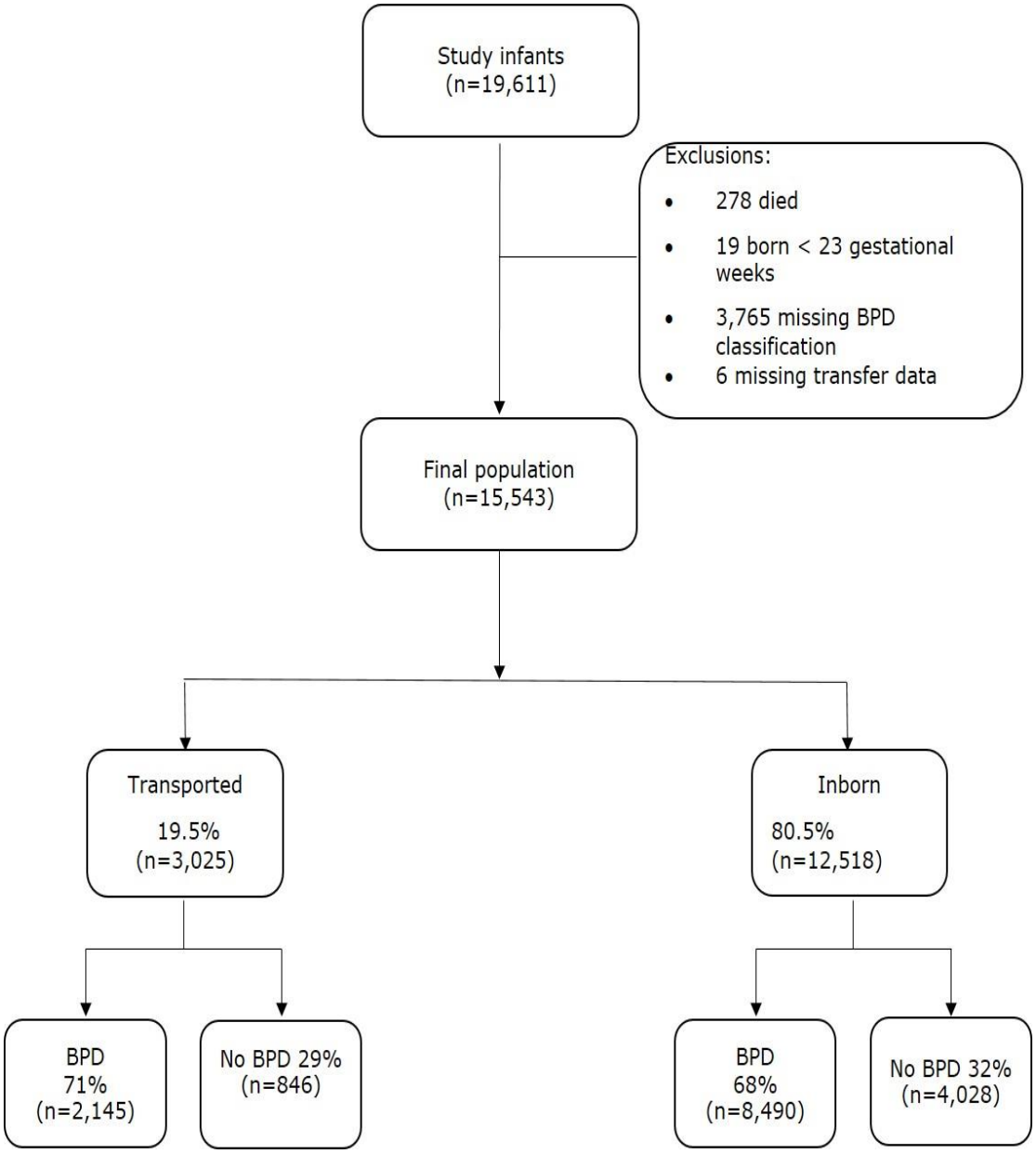


Figure 3.1 Flowchart of included infants born with gestational age 23 to 28 weeks in England and Wales during the period from 2010 to 2017 and prevalence of bronchopulmonary dysplasia (BPD)

3.4.2.1. Infant characteristics

The characteristics of extremely preterm infants included in the study are shown in Table 3.3. Based on transport status, both groups had similar birth weights and gender distribution. However, the gestational age comparison showed more preterm infants were transported.

Antenatal characteristics also revealed that transported infants were more likely to born to mothers who smoked during pregnancy and did not complete the antenatal steroid course. By contrast, transported infants had a significantly lower rate of maternal preeclampsia, chorioamnionitis, IUGR, multiple pregnancy and delivery by C-section (Table 3.3). Furthermore, neonatal characteristics showed that transported infants had lower Apgar score results at 5 minutes, a higher rate of surfactant therapy, and a higher rate of at least one course of consecutive 5 days antibiotic treatments compared to inborn infants (Table 3.3).

Table 3.3. Background antenatal and neonatal characteristics of extremely preterm infants grouped by transfer status in the first 48 hours of life.

Infant characteristics	Inborn (n=12,518)	Transported (n=3,025)	P value
Gestational age in weeks, mean (SD)	26 (1.2)	25.55 (1.2)	<0.001[t]
Birthweight in g, mean (SD)	855 (190)	853 (176)	0.63[t]
Male	6,668 (53)	1,638 (54)	0.38[x]
Smoking in pregnancy	2,053 (16)	581 (19)	0.001[x]
Complete course of antenatal steroids	9,214 (74)	1,397 (46)	<0.001[x]
Chorioamnionitis	1,033 (8)	87 (3)	<0.001[x]
Intrauterine growth restriction	724 (6)	81 (3)	<0.001[x]
Multiple pregnancy	3,074 (25)	689 (23)	0.04[x]
C-section delivery	5,235 (42)	1,068 (35)	<0.001[x]
Preeclampsia	349 (3)	56 (2)	0.004[x]
Apgar score 1 minute, median (IQR)	5 (3-7)	5 (3-7)	<0.001[u]
Apgar score 5 minutes, medina (IQR)	8 (6-9)	7 (6-9)	<0.001[u]
Surfactant therapy	11,435 (91)	2,930 (97)	<0.001[x]
Required ≥1 course of 5 days or more of antibiotics	10,359 (83)	2,621 (87)	<0.001[x]
Surgically treated PDA	707 (6)	188 (6)	0.89[x]

Data presented as n (%) unless otherwise stated; n: number; IQR: interquartile range; SD: standard deviation; PDA: patent ductus arteriosus
[t] independent t-test; [x] Chi squared test; [u] Mann-Whitney test.

3.4.2.2. Respiratory management

The respiratory management for transported and inborn infants is shown in Table 3.4. The number of infants requiring MV was significantly higher in the transported compared to inborn infants, as was the duration of days spent on MV. In addition, the number of infants receiving respiratory support via HF and CPAP was significantly higher for those transported compared to inborn infants.

Table 3.4 Comparison of respiratory management according to transport status within first 48 hours of life.

Respiratory management	Inborn (n=12,518)	Transported (n=3,025)	P Value
Infants requiring MV	11,914 (95)	2,998 (99)	<0.001[x]
Duration of days on MV , median (IQR)	11 (4-28)	15 (6-30)	<0.001[u]
Infants requiring HF	9,274 (74)	2,306 (76)	0.01[x]
Duration of days on HF, median (IQR)	20 (10-32)	20 (11-31)	0.65[u]
Infants requiring CPAP	11,188 (89)	2,843 (94)	<0.001[x]
Duration of days on CPAP, median (IQR)	19 (8-32)	19 (9-32)	0.02[u]
Infants requiring oxygen therapy without pressure support	10,876 (87)	2,642 (87)	0.5[x]
Duration of days on oxygen therapy without pressure support, median (IQR)	20 (10-32)	20 (11-31)	0.69[u]

Data presented as n(%) unless otherwise stated; n: number; IQR: interquartile range; MV: mechanical ventilation ; CPAP: continuous positive airway pressure ; HF: high flow
[x] chi-squared test ; U Mann-Whitney test.

3.4.3. BPD and transport status

The incidence of BPD was significantly higher among transported infants at 72% (n=2,145) compared to 68% (n=8,490) among inborn (*P*=0.001). The effect size analysis represents a very small effect of transport status on the BPD incidence (Φ value=0.03). However, a 6% difference could be a clinically significant.

Logistic regression was used to assess the association between BPD incidence and early transport for extremely preterm infants (Table 3.5). Transported infants were significantly more likely to have BPD compared to inborn infants.

Multiple logistic regression analysis revealed no significant association between early transport and BPD after adjusting for confounders (Table 3.5). Infant characteristics listed in Table 3.3 and respiratory management listed in Table 3.4 were subjected to confounder assessment as per criteria in section 3.3.8. Each confounder meeting the criteria was included in the final multiple logistic regression model.

Table 3.5 Association of transport with bronchopulmonary dysplasia (BPD) for all extremely preterm infants born 23 to 27 weeks of gestational age

Cohort	OR (95% CI)	Adjusted OR (95% CI)
All infants (n=15,543)	1.16 (1.06-1.26)	0.96 (0.85-1.09) [a]

n: number; OR (Odd ratio); CI (confidence interval).
[a] adjusted for gender, gestational age in weeks, birth weight, smoking in pregnancy, complete course of antenatal steroids, chorioamnionitis, intrauterine growth restriction, preeclampsia, multiple pregnancy, mode of delivery, Apgar score at 1 and 5 minutes, surfactant therapy, infants received at least 1 episode of 5 days antibiotic treatments, receive at least one day of respiratory support via mechanical ventilation (MV), duration of MV, receive at least one day of respiratory support via high flow (HF), receive at least one day of respiratory support via continuous positive airway pressure (CPAP), duration of CPAP.

3.4.4. Subgroups analysis for BPD at 36 weeks

3.4.4.1. Gestational and neonatal characteristics

The comparison of gestational characteristics for both subgroups revealed that the percentage of infants receiving a complete course of antenatal steroids, a diagnosis with IUGR and a diagnosis of chorioamnionitis was significantly lower among transported infants (Table 3.6).

Comparison of neonatal characteristics for 23-25 weeks infants showed that transported infants have a significant higher birth weight compared to inborn. Infants born between 26 and 27 weeks analysis revealed that transported infants had a significantly lower Apgar score in the first minute. In addition, transported infants have a higher percentage of receiving surfactant therapy and antibiotic course compared to inborn (Table 3.7).

Table 3.6 Comparison of gestational characteristics according to transport status within first 48 hours of life by gestational subgroups.

Gestational characteristics	Group 1 23 - 25 gestational weeks (n=5,918)			Group 2 26 – 27 gestational weeks (n=9,625)		
	Inborn (n=4,560)	Transported (n=1,358)	P value	Inborn (n=7,958)	Transported (n=1,667)	P value
Smoking in pregnancy	717 (16)	243(18)	0.09[x]	1,336 (17)	338 (20)	0.001[x]
Complete course of ANS	3,436 (75)	581 (43)	<0.001[x]	5,778 (73)	816 (49)	<0.001[x]
Chorioamnionitis	510 (11)	50 (4)	<0.001[x]	523 (7)	37 (2)	<0.001[x]
Intrauterine growth restriction	94 (2)	13 (1)	<0.01[x]	630 (8)	68 (4)	<0.001[x]
Multiple pregnancy	1,107 (24)	266 (20)	<0.001[x]	1,967 (25)	423 (25)	0.56[x]
C-section delivery	1,067 (23)	289 (21)	0.07[x]	4,168 (52)	779 (47)	<0.001[x]
Preeclampsia	62 (1)	9 (1)	0.04[x]	287 (4)	47 (3)	0.11[x]

Data presented as n (%); n: number; ANS: antenatal steroid; C-Section: Caesarean section.
[x] chi-squared test.

Table 3.7 Comparison of neonatal characteristics according to transport status within first 48 hours of life by gestational subgroups.

Neonatal characteristics	Group 1 23 - 25 gestational weeks (n=5,918)			Group 2 26 – 27 gestational weeks (n=9,625)		
	Inborn (n=4,560)	Transported (n=1,358)	P value	Inborn (n=7,958)	Transported (n=1,667)	P value
Gestational age in weeks, mean (SD)	24.4 (0.7)	24.4 (0.7)	0.001[t]	26.6 (0.5)	26.5 (0.5)	<0.001[t]
Birthweight in g, mean (SD)	709 (118)	738 (114)	<0.001[t]	938 (173)	946 (161)	0.06[t]
Male	2,355 (52)	714 (53)	0.55[x]	4,313 (54)	924 (55)	0.36[x]
Apgar Score 1 min, median (IQR)	5 (3-6)	4 (3-6)	0.11[u]	6 (4-7)	5 (3-7)	<0.001[u]
Apgar Score 5 min, median (IQR)	7 (6-8)	7 (5-8)	<0.01[u]	8 (7-9)	8 (6-9)	<0.001[u]
Surfactant therapy	4,363 (96)	1,329 (98)	0.05[x]	7,072 (89)	1,601 (96)	<0.001[x]
Required ≥1 course of 5 days or more of antibiotics	4,184 (92)	1,264 (93)	0.11[x]	6,175 (78)	1,357 (81)	0.001[x]
Surgically treated PDA	489 (11)	146 (11)	0.85[x]	218 (3)	42 (3)	0.21[x]

Data presented as n(%) unless otherwise stated; n: number; IQR: interquartile range; SD: standard deviation; PDA: patent ductus arteriosus
[x] chi-squared test; [t] independent t-test; [u] Mann-Whitney test.

3.4.4.2. Respiratory management

The number of infants receiving at least one day of respiratory support via MV and CPAP was significantly higher among transported infants in both gestational subgroups. In addition, duration of MV was significantly longer among transported infants born at 26 and 27 gestational weeks compared to inborn. Moreover, the number of infants receiving at least one day of HF was significantly higher among transported infants born at 26 and 27 gestational weeks compared to inborn (Table 3.8).

Table 3.8 Comparison of respiratory management according to transport status within the first 48 hours of life by gestational subgroups.

Respiratory management	Group 1 23 - 25 gestational weeks (n=5,918)			Group 2 26 - 27 gestational weeks (n=9,625)		
	Inborn (n=4,560)	Transported (n=1,358)	P value	Inborn (n=7,958)	Transported (n=1,667)	P value
Number of infants on MV	4,518 (99)	1,357 (100)	0.001[x]	7,396 (93)	1,641 (98)	<0.001[x]
Duration of days on MV, median (IQR)	28 (14-44)	27 (15-43)	0.62[u]	6 (2-14)	8 (4-16)	<0.001[u]
Number of infants on HF	3,533 (77)	1,046 (77)	0.72[x]	5,741 (72)	1,260 (76)	0.004[x]
Duration of days on HF, median (IQR)	22 (12-35)	22 (12-35)	0.59[x]	18 [9-30]	19 (9-28)	0.86[u]
Number of infants on CPAP	4,202 (92)	1,294 (95)	<0.001[x]	6,986 (88)	1,549 (93)	<0.001[x]
Duration of days on CPAP, median (IQR)	25 [13-37]	24 [13-36]	0.12[u]	15 [6-28]	16 [7-28]	<0.07[u]
Number of infants on oxygen therapy	4,056 (89)	1,193 (88)	0.26[x]	6,820 (86)	1,449 (87)	0.19[x]
Duration of days on oxygen therapy, median (IQR)	22 (12.5-33)	21 (12-33)	0.15[u]	19 (9-31)	19 (10-30)	0.91[u]

Data presented as n(%) unless otherwise stated; n: number; IQR: interquartile range; MV: mechanical ventilation; CPAP: continuous positive airway pressure ; HF: high flow
[x] chi-squared test ; [U] Mann-Whitney test.

3.4.4.3. BPD by gestational subgroups

BPD was significantly lower for transported infants with a gestational age 23 to 25 weeks compared to inborn. The effect size analysis showed very small effect of transport status on BPD incidence (Table 3.9). In contrast, analysis of infants born at 26 and 27 weeks gestation revealed BPD was significantly higher in transported infants compared to inborn. The effect size also presented very small effect of transport status on BPD incidence (Table 3.9).

Table 3.9 Comparison of bronchopulmonary dysplasia (BPD) by subgroup gestational age according to transfer status

Cohort	Inborn	Transported	P value	Effect size
23-25 weeks (n=5,918)	3,855 (84.5)	1,108 (82)	0.01[x]	0.03 [Φ]
26-27 weeks (n=9,625)	4,635 (58)	1,037 (62)	0.003[x]	0.03 [Φ]
Data presented as n (%); n: number. [X] Chi-squared test; Φ: Phi coefficient value				

An analysis was performed using logistic regression models to find the association between BPD and early transport according to gestational age. Transported infants born 23 to 25 gestational weeks were significantly less likely to have BPD. In contrast, infants born at 26 and 27 weeks gestational age and transported were at a significantly increased risk of BPD compared to inborn infants. However, the adjusted odds ratio found no significant association between early transport and BPD incidence after adjusting for the confounders in both subgroups (Table 3.10).

Table 3.10 Association of transport with bronchopulmonary dysplasia (BPD) by gestational subgroups

Cohort	OR (95% CI)	Adjusted OR (95% CI)
23-25 weeks (n=5,918)	0.81 (0.69-0.95)	0.93 (0.77-1.13) [a]
26-27 weeks (n=9,625)	1.18 (1.06-1.31)	1.01 (0.87-1.16) [b]

n: number; OR (Odd ratio); CI (confidence interval)
[a] adjusted for gender, gestational age, birth weight, complete course of antenatal steroids, chorioamnionitis, intrauterine growth restriction, preeclampsia, multiple pregnancy, Apgar score at 5 minutes, at least one day of respiratory support via mechanical ventilation (MV), and at least one day of respiratory support via continuous positive airway pressure (CPAP).
[b] adjusted for gender, gestational age, birth weight, maternal smoking, complete course of antenatal steroids, chorioamnionitis, intrauterine growth restriction, mode of delivery, Apgar score at 1 & 5 minutes, surfactant therapy, at least 1 episode of 5 days antibiotic treatment, at least one day of respiratory support via MV, duration of MV, at least one day of respiratory support via high flow (HF), and at least one day of respiratory support via CPAP.

3.4.5. Effect of transport status on mortality

The eligible population included 19,586 extremely preterm infants for the mortality analysis. Of these, 3,875 were transported and 15,711 infants were inborn, as illustrated in mortality flow diagram (Appendix 4). The neonatal characteristics for infants included in the neonatal mortality analysis were attached in Appendices 5 and 6.

Mortality among transported infants was 23% (n=898) which was significantly higher than inborn infants at 21% (n=3,339) (P<0.01). The effect size analysis produced a very small effect of transfer status on mortality rate among extremely preterm infants (Φ value =0.02). However, a 9% difference is likely to be clinically important.

3.4.6. Effect of transport status on the duration of MV before extubation

The median number of days to the first extubation was significantly longer in the transported compared to inborn infants (7 [3-20] vs 5 [2-19], p<0.001). The effect size value represents medium effect of transport status on the duration of MV before the first extubation (r=0.1).

3.5. Discussion

The present study aimed to evaluate the association between early neonatal transport, within the first 48 hours of life, and the incidence of BPD for extremely preterm infants. The result of this study showed that the incidence rate of BPD and the mortality rate for extremely preterm infants was statistically increased in transported compared to inborn infants. Moreover, the subgroup analysis revealed that transported infants born at 23 to 25 weeks of gestation are at less risk of developing BPD compared to inborn infants, and transported infants born at 26 or 27 weeks gestation are at higher risk of developing BPD compared to inborn infants. Despite the observed significant difference in the BPD rate, there were no significant association between early inter-hospital transport and BPD incidence after the adjustment for major confounding factors.

3.5.1. Early infant transport and BPD

The current study is the first to explore the association between early transport within the first 48 hours of life and the incidence of BPD in England and Wales. The univariate analysis of BPD among extremely preterm infants is consistent with previous studies [69, 179] showing an increased risk of developing BPD among transported infants. However, these two studies did not demonstrate causation between inter-hospital transport and BPD.

The present study investigates the possibility that early transport itself may contribute to the development of BPD among extremely preterm infants as this study adjusts for antenatal, postnatal and respiratory risk factors. The findings are similar to a previous Canadian study that compared the outcome of infants transported within first 4 days of life to inborn ones. This study demonstrated that a significantly higher crude incidence of BPD in the transported infants less than 27 weeks of gestation at 48% compared to 42% among inborn and but this was non-significant after adjustment (aOR 1.5 (95% CI 0.9-2.7)) [83]. In addition, a secondary analysis of the Neopain trial (i.e. study on the effect of morphine in

ventilated preterm infants born 23 to 32 weeks of gestational age) found that BPD was not associated with early transport (defined as shortly after birth) after adjusting for gestational age and treatment groups only with aOR of 0.7 (95% CI 0.4-1.2) [180]. However, both the Canadian and Neopain logistic regression models did not take into account the important role of respiratory risk factors, which are defined as significant determinants of the development of BPD in the literature. In addition, they did not explore the impact of early transport among extremely preterm below 28 weeks of gestational age.

A possible explanation for the higher incidence of BPD among transported infants might be that the difference in many of the risk factors between transported and inborn as observed in the univariate analysis. The antenatal factors that previously reported to be associated with an increase in the risk of BPD, and increase BPD among transported infants, include: higher rate of maternal smoking [181], reduced rates of full course of antenatal steroids [156], lower gestational age [147], lower Apgar score at 5 minutes [147], and a higher rate of receiving at least five days of antibiotics [161]. In addition, the respiratory risk factors that may contribute to increasing the incidence of BPD among transported infants are higher rate and the longer duration of receiving MV among transported infants [182]. It is important to note that such infants are likely to be electively intubated before transportation to ensure stability during transportation. Therefore, all of these factors separately or jointly could increase the incidence of BPD among transported infants within the first 48 hours of life compared to inborns.

3.5.2. The subgroup analysis and BPD incidence rate

Contrary to expectation, the present study found that preterm infants born at 23 to 25 weeks of gestational age, and underwent early transport, have a significantly lower incidence of BPD compared to inborn infants. This finding might be contributed to the significantly higher birth weight among transported infants, as birth weight is known to be a predictor of BPD development beside the degree of

prematurity [183]. However, the significant reduction in a complete course of antenatal steroids and the increase in the use of MV among transported infants for this subgroup might be not enough to increase the incidence of BPD (Appendices 8 - 10). Although it is difficult to fully explain the increased incidence of BPD among this subgroup, one additional explanation is the possibility of selection bias as there is a chance that unstable infants did not undergone early transport.

The subgroup of preterm infants born at 26 and 27 weeks of gestational age showed that the incidence of BPD increased significantly among transported infants. This result is opposite to infants born at 23 to 26 weeks. The striking difference between two subgroups could be explained by the presence of significant difference of BPD risk factors among 26 to 27 subgroup. These factors are the higher duration of MV, lower Apgar score at 1 minute, and more preterm among transported infant. These factors could signify BPD incidence, as discussed earlier in the full cohort.

3.5.3. Early transport and mortality

Early transport is associated with an increase in mortality among extremely preterm infants. Many previous studies of using different methodology also found that transported infants are at increased risk of death [184-187].

The higher mortality rate among transported infants may be explained by differences in several infant characteristics associated with an increased risk of death in preterm infants (Appendices 6 and 7). Factors aligning with previous studies that are associated with an increased death rate among transported infants include maternal smoking [188], reduced antenatal steroid course [189], NEC [13], antibiotic treatments (may be linked to ongoing infection) [13], lower Apgar scores [190], and lower gestational age [191].

I found no association between the mothers' comorbidities (i.e. maternal diabetes, maternal hypertension, and preeclampsia), pregnancy complications (i.e. chorioamnionitis, and IUGR) and an increased mortality rate among transported infants. Therefore, the increase in mortality among transported infants might be

related to other clinical factors as discussed before, and this might help us to understand the common cause-specific mortality in future studies.

3.5.4. Early transport and duration of MV before first extubation

My study findings are consistent with previous research that found transported infants had a longer duration of MV before first extubation with a median of 3 days compared to inborn with median of 2 days [84]. This could be because transported infants are frequently transported due to their illness severity and the need for intensive care treatments. Another factor is that transported infants were born at lower gestation age compared to inborn infants; the degree of prematurity plays a key role in determining the duration of MV as reported before [192].

3.5.5. Inter-hospital transport and morbidity

BPD did not appear to be associated with early inter-hospital transport in this study despite a significant increase in BPD. The EPICure 2 study reported that survival without significant morbidity was achieved in only 7% of infants transported in first day of life compared to 17% and 15% for infants cared in level 2 and 3, respectively [4]. Interestingly, previous studies have reported early life transport of extremely preterm infants is associated with the development of severe brain injury [84, 178]. The increasing incidence of brain injury among outborn infants may be due to conditions encountered during transportation including mechanical vibration [84]. Following a literature search, only one study was found discussing the impact of vibration on the infant's respiratory system and found an association with significant deterioration of respiratory function [85]. However, this study had several limitations discussed later. The impact of vibration encountered during ambulance transportation will be explored thoroughly in the next chapter.

3.6. Study strengths and limitations

3.6.1. Study strengths

The present study has several strengths compared to previous ones that explored the association between infant transport and BPD [83, 180]. The current study

included extremely preterm infants transferred within 48 hours of life and born at 23 to 27 weeks of gestational age; these infants are at high risk of developing BPD, as their undeveloped lungs are susceptible to injury. The present study involved more than 15,000 extremely preterm infants making one of the largest studies to date. In addition, this cohort study included all extremely preterm infants born in England and Wales between 2010 and 2017. Therefore, the findings are generalisable to many settings. The presented study used data from the NNRD that has been validated as described in second chapter.

The multivariable logistic regression models used considered the main risk factors for developing BPD. However, some confounding factors such as the use and duration of oxygen therapy were not included, as they did not satisfy the conditions of selecting the confounding factors. A sensitivity analysis using logistic regression including these risk factors is attached in Appendix 14, and no changes in the outcome were obtained.

3.6.2. Study limitations

The main limitation of the present study is the possibility of selection bias, as the most critically preterm infants who are prone not to survive the transportation within first 48 hours of life are often not transported. A further limitation of the current study was that it could not factor in evolving respiratory management over the period of the study. For example, implementation of the oxygen saturation target range between 91% and 95% that became common practice from 2014.

This study did not examine the impact of infants who transferred after the first 48 hours of life, as the aim of this study to find the association between infants transfer within the first 48 hours of life and incidence of BPD.

The nature of the retrospective studies makes them susceptible to recall bias. However, the risk of recall bias in this study is low because all data were recorded prospectively using an electronic health system that is then transferred to the NNRD database.

3.7. Conclusion

In England and Wales, early postnatal transport, within first 48 hours of life, is associated with greater mortality but is not associated with an increased risk of developing BPD in extremely preterm infants.

The duration of MV before the first successful extubation was higher among transported preterm infants; this finding may also correlate with the increase of BPD and reflect the ventilation management for transported infants.

3.8. Future work

This study has thrown up several questions in need of further investigation. There is a need to investigate why there is a significant increase in the duration of MV before first extubation among transported infants. Future studies should explore the difference in MV management strategies including the time ventilation was initiated, use of surfactant therapy, and HCPs prospective regard weaning and extubating transported infants.

It is interesting to note that transported infants have much less antenatal steroid and more surfactant therapy, but there appears to be no relationship between BPD and early transport. A more detailed analysis of these findings is warranted.

Further work is required to investigate the reduced BPD rate among transported infants born 23 to 25 weeks of gestational age, as found in the subgroup analysis. There is likely a difference in practice between neonatal units. Therefore, a study investigating the cause-specific mortality among transported infants for different units, aiming to explore the differences in clinical practice and any effect on the mortality rate that could explain the higher mortality among transported infants.

Chapter 4. Whole Body Vibration during inter-hospital transfer in early neonatal life: A rat model of lung injury

4.1. Overview

In 2018, there were approximately 15,000 neonatal inter-hospital transports in the UK [71]. It has been reported that transported preterm infants have a higher risk of developing BPD compared to inborn infants [69]. The aim of each transport is to keep infants stable. However, adverse factors such as WBV can compromise the infant's stability during transport [75].

The only study examining the impact of WBV on the respiratory system exposed rat pups to WBV of 27.4 m/s^2 and found that respiratory function deteriorated following vibration [85]. However, the vibration impulse of 27.4 m/s^2 used in this paper is ten times higher than most real-life neonatal transfers [80]. This model also used MV with vibration, and this may induce lung injury [182]. Furthermore, they highlighted the impact of vibration on surfactant protein mRNA expression and respiratory mechanics with little understanding of the inflammatory pathways involved. A more accurate model would seek to match the exposure seen in neonatal transport and explore the impact of the only WBV on lung injury.

The present study aims to explore the impact of WBV at levels experienced during inter-hospital transport, on the injury risk to the newborn lung utilising a rat model. Lung surfactant protein mRNA expression, key inflammatory mRNA expression, and lung histological appearance for rat pups were studied and compared at postnatal day 4 and 7 of age.

4.2. Study aim and hypothesis

4.2.1. Study aim

To assesses the impact of WBV, as experienced during ambulance transportation, on lung injury in a new rat model.

4.2.2. Study hypothesis

The vibration observed during neonatal inter-hospital transfer can have a deleterious effect on the neonatal lung.

4.3. Materials and methods

4.3.1. Theoretical model

Lung injury remains common in preterm infants, contributing to an increase in respiratory morbidity such as BPD and neonatal mortality [41]. Lung inflammation is the main mechanism of lung injury [41].

Extreme and very preterm infants are born at immature phases of lung development, i.e. the late canalicular phase for infants born at 24-26 gestational weeks and the early or mild saccular phase for those born at 27-32 gestational weeks [24]. After delivery, the immature lung will be exposed to several postnatal environmental factors that could cause injury [193]. The immature lung is susceptible to injury due to a deficient in oxidative stress and inflammatory response caused by several factors such as mechanical insults [193].

4.3.1.1. Lung inflammatory response

The lung inflammatory response is a homeostatic mechanism that is triggered to overcome insults and to maintain body integrity [194]. The innate immune system triggers instant, non-specific, immune responses against molecules from injured tissues [195, 196]. These molecules, known as damage associated molecular patterns (DAMPs), are endogenous danger signals typically placed intracellularly and released as result of tissue injury. DAMPs can trigger innate immune responses during sterile inflammation, as well as potentially triggering inflammatory responses *in vitro* and/or *in vivo* [196].

Upon tissue injury and the release of DAMPs, the body monitors for the presence of harm [195, 197]. This can be performed by specific receptors known as pattern recognition receptors (PRRs). There are several known families of PRRs, including the Toll–like-receptor (TLR) family. TLRs are Type I transmembrane glycoproteins on the cell surface or in the intracellular membrane. There are 11 human and 13 rodent TLRs that have been identified [195, 198].

TLR4 is a key member of TLR pattern recognition receptors, located at the cell surface and involved in the initial activation of the immune system [195, 198]. It senses DAMPs and activates the canonical nuclear factor kappaB (NFkβ) pathway, which is responsible for the production of proinflammatory cytokines, chemokines, and other inflammatory mediators [199].

The common cellular and molecular mediators involved in lung inflammation include interleukin 1B (IL1β), tumour necrosis factor α (TNFα) [41], transforming growth factor β (TGFβ) [200], and monocyte chemoattractant protein-1 (MCP1) [201]. The proposed method to investigate the impact of vibration in lung inflammation is illustrated in Figure 4.1.

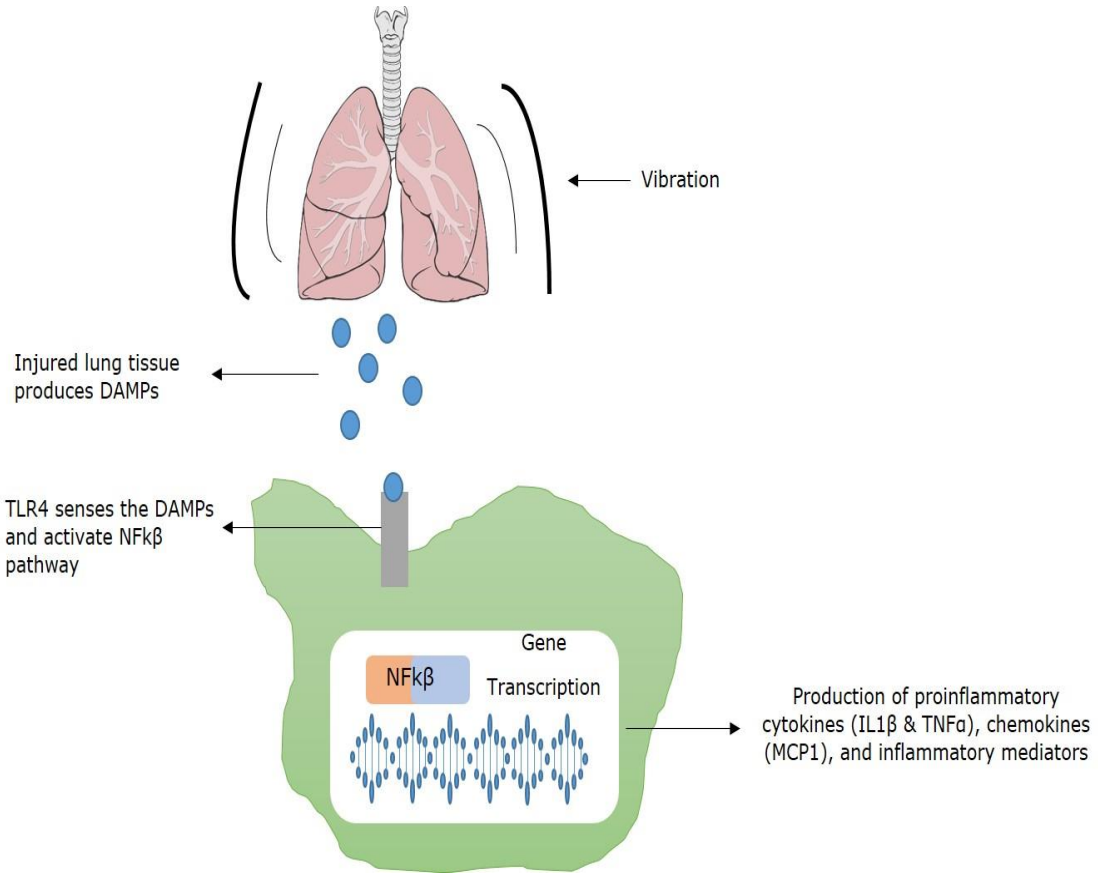


Figure 4.1 Proposed method to investigate the impact of vibration on lung inflammatory response.

4.3.1.2. Proinflammatory Markers

4.3.1.2.1. Interleukin 1β (IL1β)

IL1β is one member of the IL1 cytokine family. IL1β cytokine is not commonly expressed in healthy cells or tissues; it is induced in cells after the activation of pattern recognition receptor i.e. (TLR) by damaged tissues. This causes the accumulation of the protein intracellularly [202]. In the rat model, the induction of IL1β in the lung triggers inflammation characterised by the infiltration of neutrophils and macrophages [203]. Moreover, IL1β aggravates mucin production, disturbs alveolar septal elastin fibres, and increases tissue fibrosis formation in the airway walls.

4.3.1.2.2. Tumour necrosis factor alpha (TNFα)

TNFα is the most commonly studied member of the TNF superfamily [204]. TNFα is a pleiotropic cytokine produced primarily by macrophage and alveolar macrophages [205]. Under normal conditions, the TNF family is known to have biological functions that organise innate immunity, cell differentiation, and organogenesis [204]. TNFα is induced after the activation of the TLR4 – NFKβ pathway [206]. TNFα is one of the most abundant early proinflammatory cytokines and is considered a key early mediator of acute lung injury [204, 205].

4.3.1.2.3. Monocyte chemoattractant protein 1 (MCP1)

MCP1 was the first discovered and best described chemokine [207]. It is produced by several cell types, including the endothelial, fibroblast, epithelial, smooth muscle, and monocytic cells [208]. During lung inflammation, MCP1 expression and protein production is increased [209] acting as a proinflammatory mediator, playing a critical role in attracting monocytes to the site of inflammation [210].

4.3.1.2.4. Transforming growth factor beta 1 (TGFβ1)

TGFβ1 is essential for lung development and homeostasis [211]. During lung inflammation, TGFβ1 is upregulated and aids the resolution of lung injury during the late phase of lung repair and fibrotic tissue formation [200]. TGFβ1 also

increases the permeability of endothelium and epithelium, which leads to lung oedema [200].

4.3.1.3. Surfactant proteins and lung inflammation

Surfactant is the major component of the lung, which is responsible for decreasing alveolar surface tension [27]. There are four types of surfactant proteins: SPA, SPB, SPC, and SPD, which all described earlier in the introduction chapter [27].

SPA is an important factor responding to inflammation and aids in inflammation regulation and the repair of lung tissue after injury [212]. SPD exerts anti-inflammatory properties and limits the inflammatory response after lung tissue injury, SPD has been involved in the development of BPD [213]. In the rat model, exposure to mechanical stretch could increase the expression of SPB and SPC [214].

4.3.2. Use of rat model to study lung injury

Animal models aid studying hypotheses generated from human studies or observations and contribute significantly to enhancing our knowledge of the potential mechanisms causing lung injury [215]. The ideal animal model should have the same pathological manifestations and consequences of a human lung injury [216].

Newborn rodents develop lung inflammation and injury responses that are similar to those of human infants [217, 218]. As such, rodent models have been developed to study preterm lung disease associated with oxygen administration, MV and inflammation [217, 218].

One of the advantages of using a rat pup model is that they are born at the lung development stage that is equivalent to that of an extreme preterm infant [219]. A full-term rat, born at 22 days of gestational age, is at the saccular lung development stage [219]. Despite the lung anatomical immaturity in term rats, they are physiologically mature, and the ability of the lungs to function normally is

because of their surfactant production at birth [219]. Furthermore, all four surfactant proteins (SPA, SPB, SPC, and SPD) are detected in Type II pneumocytes [219]. Another advantage of using rat is the commercial availability of tools and products such as PCR (polymerase chain reaction) primers that required for the analysis [219].

Newborn rats are small, and like the preterm human infants, it is unclear the dose of WBV to use and how this relates to adult exposure. The use of larger animal models such as the preterm lamb are possible. However, the preterm lamb models require intensive positive pressure ventilation and oxygen therapy where could interfere with lung injury development [220]. Despite the size disadvantage mentioned above, rat models of BPD are the most frequently used model in a research context and considered as the stepping-stone to larger animal models [221].

4.3.3. Procedural and legal declaration

The rat experiments were conducted in 2016 at the Bio Support Unit, Queens's Medical Centre at The University of Nottingham. This work aimed to investigate the impact of vibration, as experienced during neonatal ambulance transportation, on the newborn rat. All the procedures involving in this study were conducted with UK Home Office approval and in accordance with the UK Animals (Scientific Procedures) Act of 1986 (Project license: PPL 40/3560). These studies received ethical approval from the ethics committee of the University of Nottingham.

All laboratory procedures used were in compliance with the UK Health and Safety Executive's Control of Substances Hazardous to Health (COSHH, SI No.1657, 1988) and risk assessment guidelines. Disposable nitrile gloves and laboratory coats were worn during laboratory procedures. All material suppliers' details can be found in Appendix 15. Application of the methods were as recommended by the manufacturers or as modified by Dr Ian Bloor and myself.

All laboratory experiments were performed in the Division of Child Health, Obstetrics and Gynaecology, School of Medicine, the University of Nottingham during the period of January 2017 to May 2019.

4.3.4. Rat model of WBV

4.3.4.1. Rat pups

Sprague-Dawley rat pups were used, with timed pregnancy to ensure the accuracy of the delivery date. Rat pups were birthed in-house to avoid early postnatal transport, with the age of postnatal day 4 and 7 were used as two cohorts. These two age points are equivalent to a human near and early-term infants in respect of lung development [219, 222, 223]. All animals were housed with their mothers and had free access to maternal milk and water. The environmental temperature was kept controlled at 21 °C with regulated light/dark periods to minimise stress exposure. All live animal work and dissection were performed by Dr Lara Shipley and Dr Ian Bloor.

4.3.4.2. Study design

The experimental study used a rat model to explore the impact of short-term WBV on the surfactant protein expression, initiation of lung injury cascade and anatomical structures of the lung.

4.3.4.3. Experimental procedure

Each age point cohort was divided based on gender into males and females. Each gender was then randomly divided into control and vibration group; the study design is summarised in Figure 4.2. Female pups in the vibration group were exposed to the upper range of vibration programme (high) and male pups in the vibration group were exposed to moderate levels of vibration group, both level of vibrations simulated the typical neonatal exposure observed during ambulance transfer (detailed later) [80].

The differences in vibration level between genders was used to minimise variables that could impact on the intervention. Significant respiratory disease is found more frequently in male infants. Infant male lung structure and function seems to have a delay in surfactant production, lung maturation and lung response to injury [224-228]. In the rat model, it is known that oestrogen hormone could accelerate lung development while testosterone has an inhibitory role [227].

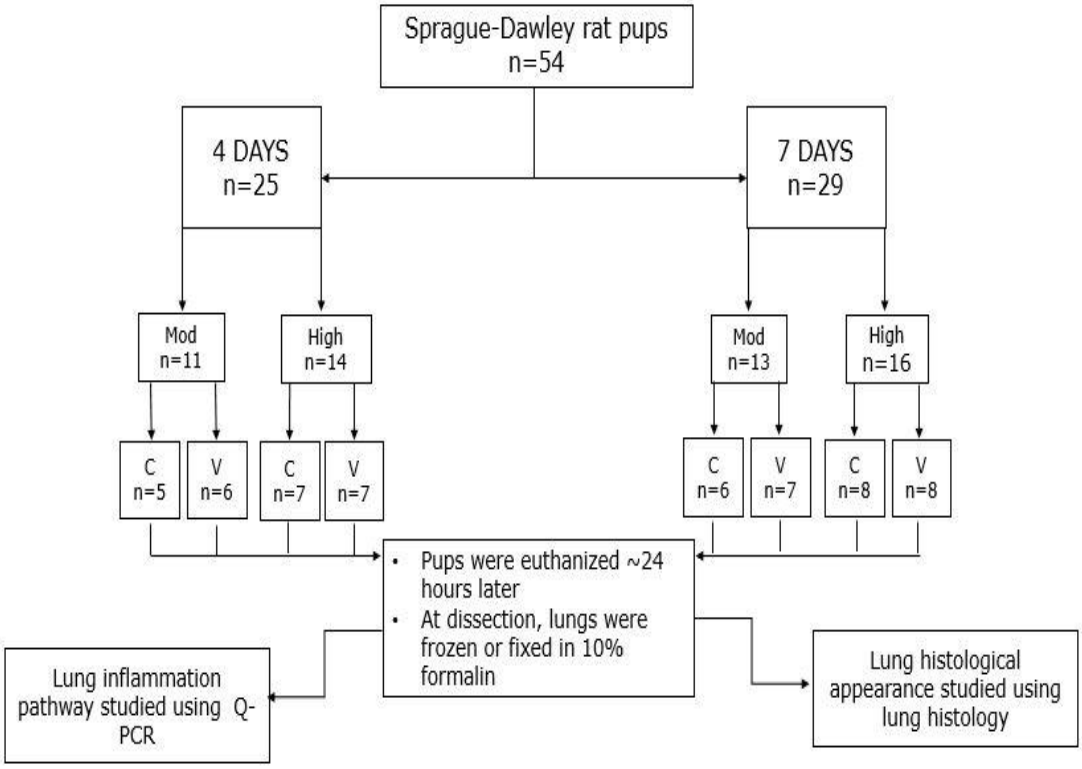


Figure 4.2 Whole body vibration and lung injury study flowchart.

Mod represents level of vibration = 0.9m/s^2, High represents level of vibration = 2.0 m/s^2. C represents control group and V represents vibration group.

On the experimental day, rat pups were separated from their mother's and taken into a room with controlled temperature at 21 °C. Vibration exposure rat pups were placed, none anaesthetised, into a segmented box fixed to a vibration platform (Multi-function 3D rotator PS-M3D, grant-bio, UK), and control rat pups were placed in a container within the same room, for the duration of the 90 minute study.

Both vibration compartments and control container contained a small amount of bedding material to reduce stress due to maternal separation (Figure 4.3).

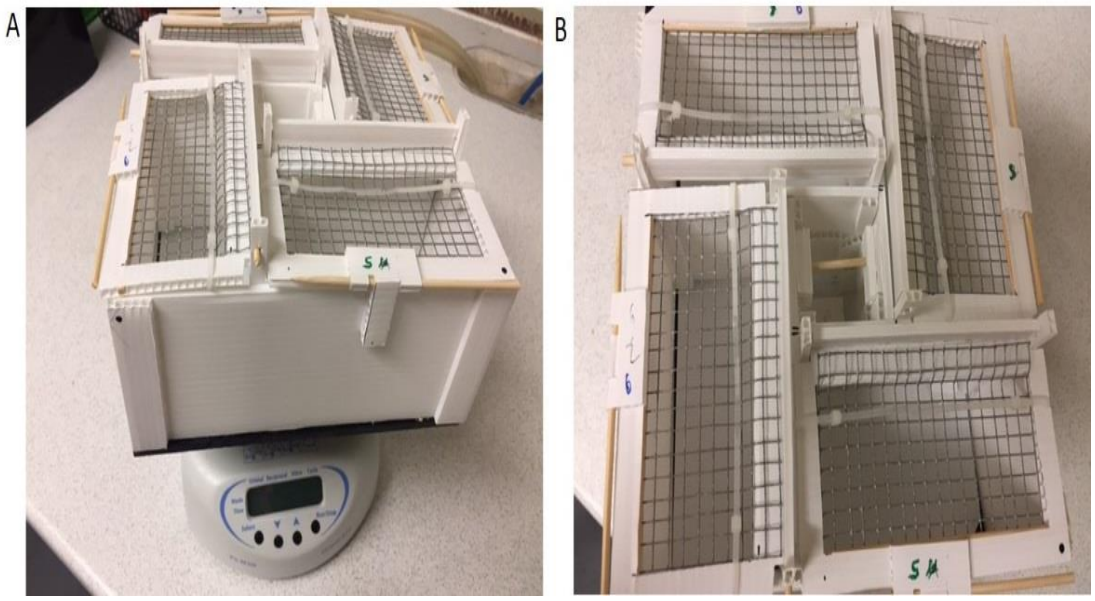


Figure 4.3 A) vibration platform with attached holding box B) overhead view of the holding box with four separate chambers

Accelerometers (Triaxial, model 3093B, Dytran, Doncaster, UK) were placed on marked positions either side of the vibration box to measure WBV. The amount of WBV was measured using a CE marked vibration level meter (model SV106, Svantek, Warsaw, Poland). The vibration programme simulated typical WBV exposure during ambulance transfer as determined by Blaxter *et al* [5]. Blaxter utilised a customised accelerometer. Therefore, the Svantek meter was compared to the customised sensor. Four-vibration programs were tested to assess vibrations and ensure they matched those observed by Blaxter (Figure 4.4).

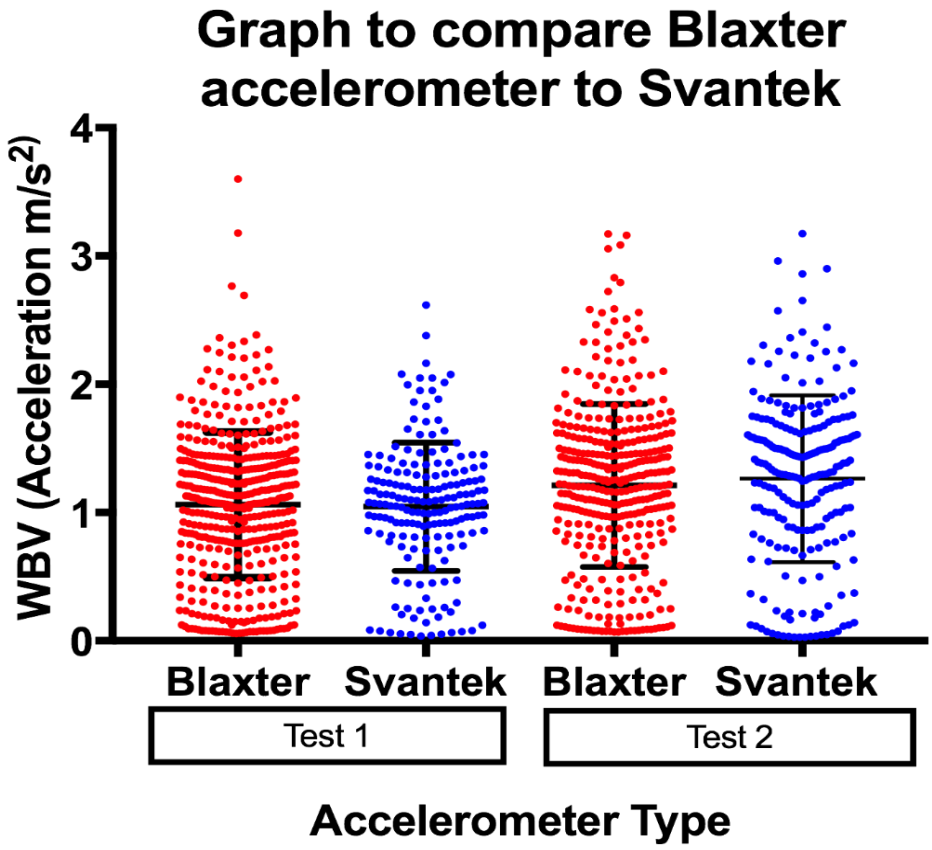


Figure 4.4 comparison between the customised accelerometer by Blaxter and Svantek vibration meter with Dytran triaxial sensors.

Note: Each plot is a measure of vibration averaged over 10 seconds throughout the programme. Error bars show the mean and standard deviation.

Statistical comparison using paired t-tests revealed that there was no statistically significant difference between the two devices in the measurement of the WBV. The vibration platform has three modes of determining movement direction. Each mode was tested by increasing the intensity gradually to define the maximum value of acceleration and the deviation from the mean. A 90-minute program was created; this is comparable to an average ambulance trip as determined by Blaxter *et al* [80]. Each program has short interval breaks to mimic a real ambulance journey with brief stops. During testing, the vibration box was weighted with masses equivalent to rat pups. The weight was used to mimic the mass of a rat pup in the box as vibration properties change with added mass and distribution in the box. Therefore if we did not, the vibration measurement recorded would not be the

same as that the rats would be exposed too while setting the vibration program, so it enhances the accuracy of setting the correct amount in the vibration program. The moderate vibration program runs for 86 minutes duration at an average WBV of 0.9 m/s^2 intensity and high vibration program runs for 90 minutes at an average WBV of 2 m/s^2 intensity. The moderate program was slightly shorter due to their need of lower average WBV exposure which decreased the length of the pre-set motion cycle utilised on the vibration platform.

At the end of the allocated programme both control and vibrated pups were clearly marked with permanent pen to allow for later identification and placed back with their mothers for 24 hours.

4.3.4.4. Post-mortem lung tissue process

Twenty-four hours after the study, all rat pups were humanely euthanised via cervical dislocation and decapitation as per schedule 1 Animal (Scientific procedure) Act 1986. The lungs (and other organs/tissues) were then dissected and frozen by liquid nitrogen and stored in a -80°C freezer or fixed in 10% formalin for histological analysis purpose. All of the samples were labelled with animal ID.

All the experiment to this stage conducted by Dr Ian Bloor and Dr Lara Shipley.

4.3.4.5. Ribonucleic acid extraction

Ribonucleic acid (RNA) extraction was conducted using a modified single-step acidified phenol-chloroform homogenisation method [229] with RNA extraction kits and instruments. RNA extraction began by lysing and homogenising the desired samples in phenol and guanidine thiocyanate mono-phase solution; this step causes protein denaturation and inhibition of RNAase activity [230]. Adding chloroform results in three layers being formed, the protein dissolved in the bottom layer, DNA dissolved in the middle layer, and RNA only dissolved in top supernatant aqueous layer [229, 230]. Following the formation of the layers, the uppermost (i.e. aqueous) layer will be aspirated and treated with ethanol to allow for proper

binding conditions and then using RNA centrifugation column where total RNA (≥100µg) binds to the column membrane. Following ethanol and guanidium salt-based buffer washes, to remove any contaminants, the final elution of RNA is performed using nuclease-free water.

The amount and the purity of extracted RNA was checked using spectrophotometry. Nucleic acid absorbs UV light at a wavelength of 260nm, protein absorbs light at wavelength 280nm, and thus RNA concentration can be estimated relatively using wavelength absorption [231].

4.3.4.5.1. RNA extraction procedure

Prior to the start of the RNA extraction procedure, laboratory benches and instruments were cleaned and sterilised utilising RNAseZap (Ambion, Foster City, California, USA). The lung tissues were identified by the animal ID and removed from -80°C freezer then they were transferred into dry ice box for RNA extraction. Approximately 60 mg of lung tissue was chopped and treated with 500 µl ml TRI reagent (Sigma-Aldrich, St. Louis, Missouri, USA) and homogenised on dispomix homogeniser (Medic Tools, Zurich, Switzerland) at 4000 g for 45 seconds. The homogenate was pipetted using sterile pipette tips into a labelled sterile 1.5ml Eppendorf tube. 100µl of chloroform (Fisher Scientific, Leicestershire, UK) was added to each sample, mixed by vortex, and incubated for 10 minutes at room temperature.

Following the incubation, samples were centrifuged at 12,000g for 15 minutes at 4°C. Following this step, three layers were obtained, the upper aqueous layer was carefully aspirated with a sterile pipette and transferred into labelled genomic deoxyribonucleic acid (gDNA) eliminator column and centrifuged at 8,000g for 1 minute at room temperature. An equal volume of 70% ethanol was added to each sample and mixed by vortex.

RNA extraction and purification were conducted using the RNeasy plus Mini extraction kit (Qiagen, Crawley, West Sussex, UK). RNeasy spin columns were labelled and 700µl of each sample aspirated and transferred into the column, centrifuged at 8,000g for 15 seconds and the through flow discarded. This step was repeated until the total sample had passed through the column. Total RNA binds to the RNeasy membrane, contaminants are washed away as per the manufacture instructions, and highly pure RNA is eluted in RNAse free water.

To measure the RNA concentration and check the purity, the NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) spectrophotometer was used. The optical density ratio (260:280 nm) was >2.0 for all samples (Appendix 16). All RNA samples were stored at -80°C freezer for further analysis.

4.3.4.6. Reverse transcription polymerase chain reaction

4.3.4.6.1. The principle of RT-PCR

The reverse transcriptase PCR (RT-PCR) is a process with ability to transcribed RNA into double stranded complementary deoxyribonucleic acid (cDNA) for later amplification for gene analysis.

RT-PCR involved using template mRNA, a DNA primer sequence that anneals to its complimentary sequence of mRNA then elongation and transcription occurs under enzymic reaction resulting in formation of the cDNA molecule. Figure 4.5 illustrates the principle of RT-PCR.

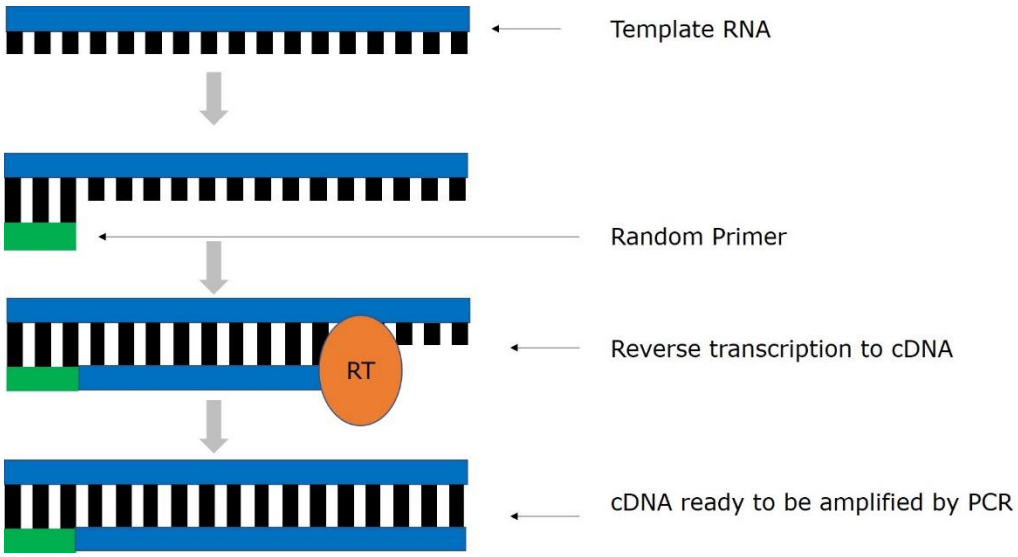


Figure 4.5 Diagram of reverse transcription of polymerase chain reaction, a random primer annealed to single stranded ribonucleic acid (RNA) and sequence is elongated under reverse transcriptase (RT) enzymic reaction to create complementary deoxyribonucleic acid (cDNA). cDNA can be amplified by polymerase chain reaction PCR.

4.3.4.6.2. RT-PCR procedure

This procedure was performed using High Capacity RNA to cDNA kits (Applied BioSystems, Warrington, Cheshire, UK).

The RNA sample was thawed on ice at 4°C with a 0.2 ml free Eppendorf tube prepared and labelled for each sample. To ensure transcription efficiency, two negative controls i.e. no template control (NTC) and no reverse transcriptase control (NRTC) 0.2ml tubes were also prepared.

For each sample, 1µg of total RNA template (depending in the starting amount of RNA) was mixed with 10µ of 2X RT buffer and 1 µl of 20X RT enzyme mix, and the volume was brought up to 20 µl by adding nuclease-free water. Details regarding the preparation of controls are shown in Table 4.1.

Table 4.1 Details of the reagents and quantities used to prepare the no template control (NTC), and no reverse transcriptase control (NRTC).

	NTC (µl)	NRTC (µl)
RNA	0	9
2X RT buffer	10	10
20X RT enzyme mix	1	0
Nuclease-free water	9	1
Total volume	20	20

The sample tubes were then centrifuged at 4,000g for 1 minute at room temperature and all samples were incubated in a thermal cycler (TechEn., Milford, MA, USA) for 60 minutes. The reaction mix is heated to 95°C for 5 minutes and then held at 4°C for primer elongation. After completion, the template cDNA was diluted to 1:10 using nuclease free water and stored at -20°C until further analysis.

4.3.4.7. Quantitative Polymerase Chain Reaction (qPCR)

4.3.4.7.1. The principle of qPCR

The polymerase chain reaction (PCR) improves the detection and quantification of a sequence of either DNA or mRNA, this quantification can be achieved at the same time *in vitro* without any additional steps of analysis such as gel electrophoresis or northern blotting. PCR can reduce the time taken for the analysis, the cost and the risk of contamination [232].

Quantitative polymerase chain reaction (qPCR) detects and quantifies cDNA by measuring a fluorescent signal emitted when a fluorogenic reagent binds to cDNA. The greater the emitted signal, the higher the concentration of cDNA present [233]. The qPCR instrument has a detector unit that only detects the fluorescent signal after a specified level of background fluorescent has been crossed, i.e. cycle

threshold (Ct), and the higher the number at the beginning of the reaction, the lower cycle's number required to cross Ct. Therefore, the Ct value is used as the parameter to quantify the initial quantity of target template in the sample [234]. Furthermore, as the amplification is exponential, the fluorescent signal represented is a sigmoid curve, which allows for comparison between the linear section of the curve and other experimental runs.

Prior to gene expression quantification, samples are normalised to overcome any variation in starting RNA abundance. The normalisation achieved by analysing the expression of housekeeping genes, which are expressed across all cells against the targeted genes. The relative gene expression can be calculated mathematically by finding the ratio of change in Ct value of housekeeping genes against change in Ct value of targeted genes. This method known as the comparative threshold ($2^{-\Delta ct}$), where ΔCt = [average Ct of target gene – average Ct of reference gene (of same sample)], this method assumes that the efficiency of the PCR reaction is 100% [235]. Use of TaqMan probes is preferred to increase the specificity of qPCR. The TaqMan principle depends on the presence of the specific 5'-3' oligonucleotide probe, this probe contains a reporter 5'end, which reports the fluorescent signal to the instrument, and a quencher at the 3'end, which absorbs the reporter signal unless it is permanently broken apart [236].

4.3.4.7.2. Selection of Housekeeping genes for Q-PCR

The selection of housekeeping genes as internal controls for quantification is important to enhance experimental reliability. The housekeeping genes should have very little variation among the sample set to correct the variation between samples [237, 238]. In order to optimise the measurement of mRNA expression level, it is recommended to use the geometric mean of multiple reference genes [237, 239].

The housekeeping genes ribosomal 18s (r18s), ribosomal protein L13A (RPL13A), ribosomal protein S29 (RPS29) and TATA box binding protein (TBP) have all been validated in previous studies as suitable reference genes [240, 241]. In addition, a

new housekeeping gene tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ) was used to assess if stable in the rat lung. The stability of the five housekeeping genes were analysed using geNorm software v3.5 (Primer Design Ltd, Southampton, UK). The geNorm software uses an algorithm to calculate the geometric mean and pairwise variation of each housekeeping genes in order to determine the normalisation factor and the expression stability value (M) for each sample among all five housekeeping genes. The two most stable genes with the lowest M value for normalisation should be used [237, 239], RPL13a and RPS29, were identified as the most stable housekeeping genes and selected as reference genes for this study. Figure 4.6 illustrates the geNorm output from the five housekeeping genes used in lung mRNA analysis.

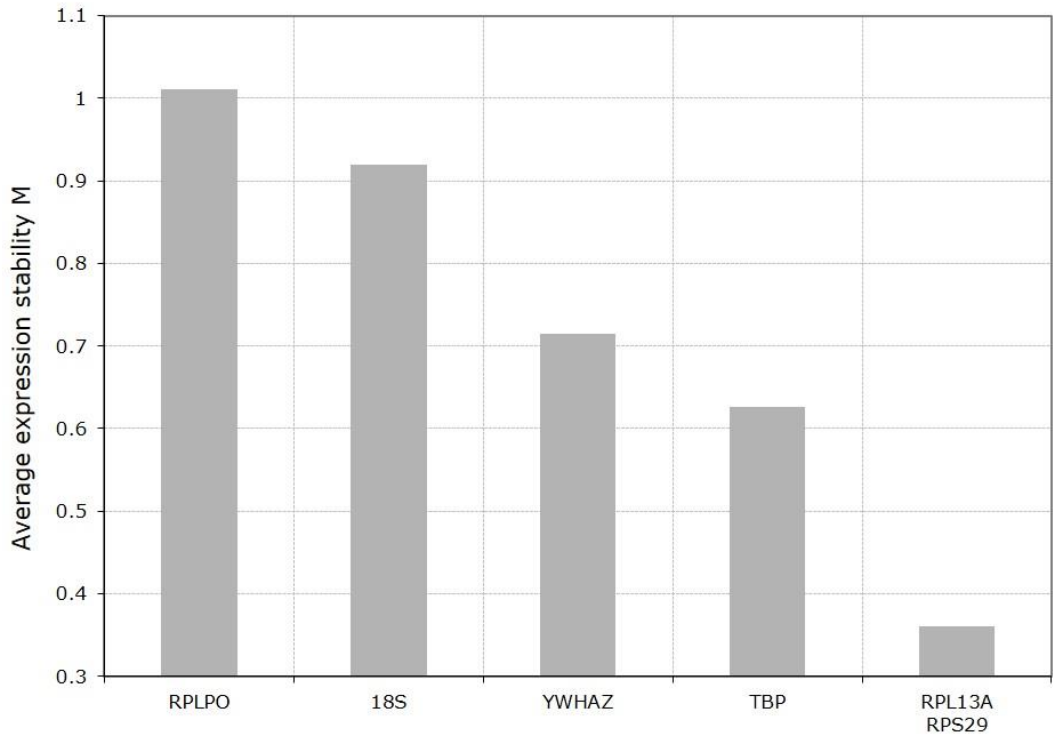


Figure 4.6 Graphical output obtained from geNorm software in analysis of five housekeeping genes.

4.3.4.7.3. qPCR reaction procedure

qPCR reactions were performed using the TaqMan method, this method does not require standards as the probes (Table 4.2) are already standardised by the manufacturer (Appendix 17, BioRad, Watford, UK). First, cDNA samples and NRTC were thawed on ice and then briefly centrifuged to collect liquid at the bottom of the tube. TaqMan gene expression probes and iTaq supermix were thawed on ice and the supermix bottle was gently swirled. To ensure experiment efficiency, samples were loaded in duplicate with negative control (nuclease-free water) and NRTC. A master mix containing iTaq Universal probes supermix, a TaqMan prime PCR probe assay, and nuclease free water were prepared, and 8µl of this mix was loaded into each well of a sterile 96 well PCR plate (Abgene; Thermo Fisher Scientific Inc.; UK). Following this, 2µl of the cDNA samples or nuclease-free water (negative control) were loaded into the corresponding wells. Negative controls were run on all plates for each gene analysed to allow for data calibration. Reaction setup and quantities of samples and reagents are listed in Table 4.3.

Table 4.2 Target probes for TaqMan

Target Gene	Gene Symbol	Organism	Assay ID	Assay Design
Surfactant Protein A	SPA	Rat	qRnoCEP0031932	Exonic
Surfactant Protein B	SPB	Rat	qRnoCEP0031431	Exonic
Surfactant Protein C	SPC	Rat	qRnoCEP0025408	Intron
Surfactant Protein D	SPD	Rat	qRnoCIP0031383	Intron
Nuclear Factor Kappa β	NFKβ	Rat	qRnoCIP0025529	Intron
Toll-like receptor 4	TLR4	Rat	qRnoCEP0024776	Exonic
Tumour Necrosis Factor Alpha	TNFα	Rat	qRnoCEP0030948	Exonic
Interleukin 1 beta	IL1β	Rat	qRnoCIP0026511	Intron
Monocyte Chemoattractant Protein 1	MCP1	Rat	qRnoCEP0031103	Exonic
Transforming Growth Factor Beta 1	TGFB1	Rat	qRnoCIP0031022	Intron

Table 4.3 TaqMan individual assay probe reaction setup

Component	Volume per reaction (µl)
Probe	0.5
iTaq Universal Supermix	5
Nuclease Free Water	2.5
cDNA sample	2

A calibrator is used across all PCR plates to normalise variation between PCR runs. Samples and the calibrator were run in duplicate to calculate the coefficient of variation within each experiment. Negative controls were run for each gene analysed

Once prepared, the PCR plate was sealed using an Abgene plate sealer and thermal seals (Alpha Laboratories; Hampshire, UK) and placed in the StepOne Plus Real Time PCR System (Applied Biosystems; Warrington, UK). The qPCR was run on the pre-set program detailed in Table 4.4.

Table 4.4 TaqMan Prime PCR Cycling Protocol

Step	Temperature	Duration	Number of Cycles
Activation	95°C	2 minutes	1
Denaturation	95°C	5 seconds	40
Annealing/Extension	60°C	30 seconds	

4.3.4.8. Histology

4.3.4.8.1. Lung Sample preparation and Histological tissue Processing

Random sections of lung tissue from each animal, stored at room temperature, were taken from 10% formalin (10% v/v formaldehyde in 0.9%w/v sodium chloride/distilled water (Fisher Scientific, Loughborough, Leicestershire, UK) for approximately two hours. Segments of each sample were placed into Histosette II (Simport, Quebec, Canada) 30mm X 27mm X 5mm cassettes and processed through increasing concentrations of six phases of ethanol dehydration from 75% to 100% (total of 6 hours, 1 hour/phase) at room temperature, followed by three phases of xylene (Fisher Scientific, Loughborough, Leicestershire, UK) (total of 3 hours, 1 hour/phase) at room temperature to clear ethanol. Samples were then immersed in three phases of paraffin wax (total of 4 hours, 1:20 hour/phase) utilising the Shandon Excelsior™ tissue wax processor (Thermo Fisher Scientific, Runcorn, Cheshire, UK) at 61 - 63C°, and left solidifying overnight to ensure fully fixation of each sample, tissue processing protocol is summarised in Table 4.5.

Following this, each tissue block was sliced at section thickness of 5 µm using a sledged microtome (Anglia Scientific, Cambridge, UK). Prior to tissue mounting, all tissue slices were rinsed in 70% ethanol and floated in a 45C° distilled water bath to stretch out the slice by surface tension. They were then mounted onto Superfrost™ plus microscope slides (Thermo Scientific™) and dried on a heated rack for at least 15 minutes to evaporate water before transferred to a drying oven at 37C° for 24 hours.

Two slides, separated by multi-tissue layers to obtain two different levels throughout each tissue block, were prepared for each subject; (54 lung samples X 2 slides = 108 total slides) for the purpose of histological staining.

Table 4.5 Protocol for processing tissues

Step	Chemical	Time
1	75% Ethanol	60 minutes
2	90% Ethanol	60 minutes
3	90% Ethanol	60 minutes
4	100% Ethanol	60 minutes
5	100% Ethanol	60 minutes
6	100% Ethanol	60 minutes
7	Xylene	60 minutes
8	Xylene	60 minutes
9	Xylene	60 minutes
10	Paraffin wax	80 minutes
11	Paraffin wax	80 minutes
12	Paraffin wax	80 minutes
Total		780 minutes (=13 hours)

4.3.4.8.2. Haematoxylin and Eosin staining

Haematoxylin and Eosin (H&E) staining are commonly used to illustrate anatomical and pathological alteration that characterise lung injury. Harris Haematoxylin is a basic aluminium salt dye with positive charge used for staining cell nucleus blue. Haematoxylin is not a stain and needs to be oxidised to haematein by mercuric oxide, which has the colour property, to bind to acidic structures in tissue, such as nucleic acid [242, 243]. Eosin is an acidic dye has the ability to bind with basic structures, named eosinophilic, it stains collagen pink and cellular cytoplasm red [242, 243].

4.3.4.8.3. H&E staining procedure

Sections were stained with H&E using a sequenced standard protocol (Table 4.6). Slides were first dewaxed in two consecutive xylene jars for 3 minutes. Secondly,

the slides were rehydrated through two stages of 100% ethanol immersion at 3 minutes for each stage, followed by a three minute stage 70% ethanol/distilled water immersion. After tissue rehydration, a distilled water rinse stage was performed for three minutes. Thirdly, The sections were nuclear-stained by immersion in a jar of Harris haematoxylin (Sigma-Aldrich) for one minute and then rinsed in running tap water for a minimum of five minutes until the water runs clear to remove all excess dye. The haematoxylin stain was regressed by placing the sections in an acid-alcohol solution (1% concentration of hydrochloric acid in 70% ethanol) for five seconds then immersed in alkaline Scott's tap water (0.2% sodium bicarbonate and 20% magnesium sulphate distilled water solution) for 90 minutes to regress haematoxylin stain further. Slides were rinsed in running tap water for five minutes and transferred to a 1% Eosin (VWR ltd) for three minutes and then rinsed in running tap water for a minimum of five minutes until the water runs clear to differentiate eosinophilic staining. Sections were then dehydrated by placing them in two stages of 100% ethanol for two minutes followed by two stages (two and three minutes) immersion of xylene to clear ethanol. Finally, the sections were mounted with microscopic cover glass using DPX mounting medium (Fisher Scientific) and left to dry overnight.

Table 4.6 Protocol for lung Haematoxylin and Eosin staining

Step	Chemical	Time
1	Xylene I	3 minutes
2	Xylene II	3 minutes
3	Absolute Alcohol I	3 minutes
4	Absolute Alcohol II	3 minutes
5	70% Alcohol (IMS)	3 minutes
6	Distilled Water	3 minutes
7	Harris' haematoxylin	1 minute
8	Running Tap Water	5 minutes
9	Acid Alcohol (1%HCL in 70% alcohol)	5 seconds
10	Scott's Tap Water	90 seconds
11	Running Tap Water	5 minutes
12	0.1% Eosin	3 minutes
13	Running Tap Water	2 minutes
14	Absolute Alcohol III	2 minutes
15	Absolute Alcohol IV	2 minutes
16	Xylene III	2 minutes
17	Xylene IV	3 minutes

4.3.4.8.4. Histological lung injury scoring system

One approach to objectively assess and quantify lung injury is based on a well-developed scoring system [244]. This scoring system quantifies lung injury based on five parameters described in Table 4.7, number of neutrophils counted in the alveolar space, number of neutrophils counted in the interstitial space, presence of hyaline membrane, presence of pink proteinaceous debris filling alveolar space and the thickening of alveolar septal.

Table 4.7 Characteristics of parameters used to score lung injury

Characteristics	Score per field		
	0	1	2
Neutrophils appear as ring shapes in alveolar space	Not shown	1-5 neutrophils shown	More than 5 neutrophils shown
Neutrophils appear as ring shapes in interstitial space	Not shown	1-5 neutrophils shown	More than 5 neutrophils shown
Hyaline membrane presents as the appearance of single eosinophilic strip of fibrin within alveolar space	Not shown	Presence of single strip	Presence of multiple strips
Proteinaceous debris filling alveolar space	Not shown	Presence of single debris	Presence of multiple debris
Alveolar septal thickness	Less than twice normal	Thickness between twice and 4 times the normal	Thickness more than 4 times the normal.

To produce the lung injury score, the sum of the five parameters shown in Table 4.7, are calculated for each field, and then are normalised to sum number of the evaluated field for each sample as per the equation:

Lung injury score= [(20 x score of neutrophils in airspace) + (14 x score of neutrophils in interstitial space) + (7 x score of hyaline membranes) + (7 x score of proteinaceous debris filling the air space) + (2 x score of alveolar septal

thickening)] / (number of fields x 100). The result is a continuous value between zero and one, where one indicate very significant injury.

4.3.4.8.5. Microscope imaging

The H&E stained slides were visualised using a Nikon 90i microscope (Nikon UK Limited, Surrey, UK) and were imaged by Volocity image software v6.1.1 (Perkin Elmer, Massachusetts, USA). The slides were visualised at a magnification of 40X, 200X, and 400X and 20 random fields were photographed for analysis and scoring using a Retiga-2000R CCD digital camera (QImaging, Surrey, BC, Canada) at 400X magnification.

The selection of random fields should have at least 50% occupied by lung alveoli, have no large airways or blood vessels, and is placed at least one field in length from other fields [244]. Twenty fields per subject were blindly scored using the three-level scoring system summarised in Table 4.7, and the blinding process illustrated in Figure 4.7.

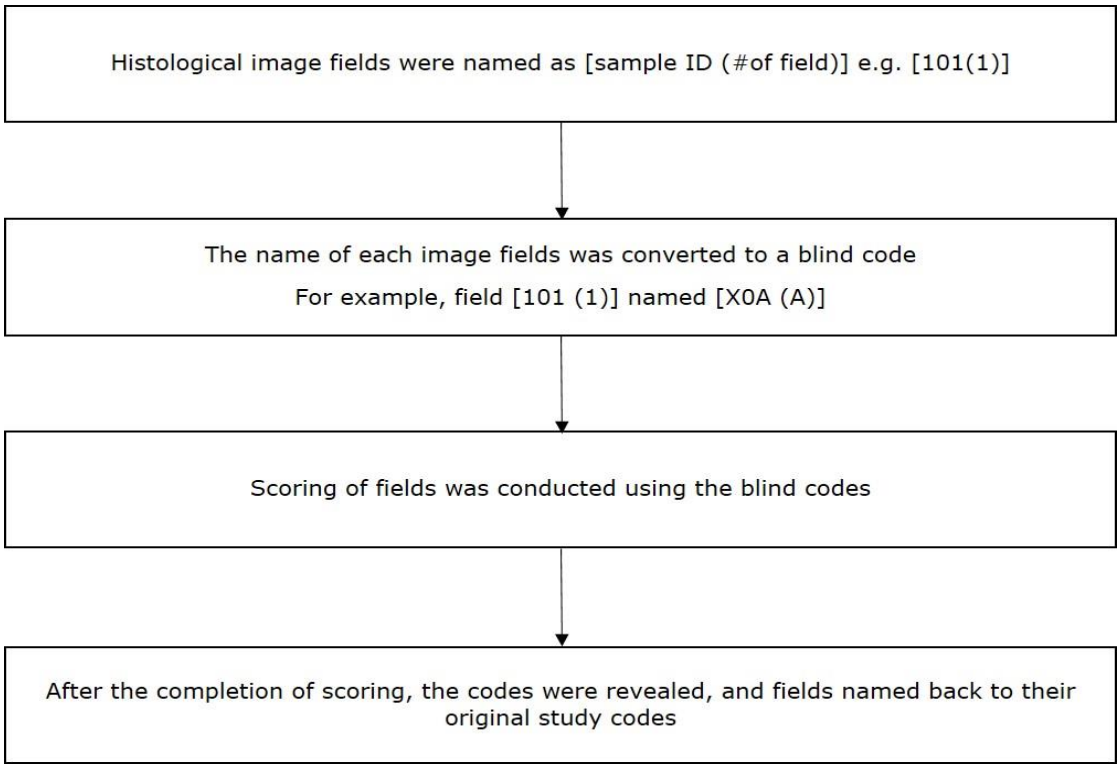


Figure 4.7 Blinding process of histological scoring for histological image fields

4.4. Statistical Analysis

The coefficient of variance for all qPCR samples run in duplicate were calculated using Microsoft Office Excel Software 2016 (Microsoft Corporation, Berkshire, UK). Duplicates with a coefficient of variance ≤5%, indicating low variance between duplicate samples, were included in the statistical analysis. All samples met this criterion.

Statistical analysis was performed using GraphPad Prism v8.0 (GraphPad Software, California, USA). Data points are presented as mean ± standard error of the mean (SEM).

Continuous data were subjected to the Shapiro-Wilk test to determine if normally distributed with significance set at $P \geq 0.05$ defining normality. Unpaired student t-test was used for normally distributed data or Mann Whitney U test for non-normally distributed.

The ontogeny expression of surfactant protein and inflammatory markers for all control groups were described first to compare control groups between the two age points.

The aim was to examine the impact of vibration on surfactant protein expression, inflammatory markers and histological degree of lung injury. Therefore, the comparison was conducted between control and vibration groups for each age point and level of vibration separately.

The results of any comparison were considered statistically significant if P value <0.05.

4.5. Results

4.5.1. The effect of postnatal age on lung surfactant proteins and inflammatory mediator's mRNA expression

The analysis of mRNA expression between the two age points i.e. postnatal day 4 and day 7, with no intervention, showed that the mRNA expression of SPA significantly reduced at postnatal day 7 compared to postnatal day 4 (0.8±0.06 vs. 1.2±0.07, respectively. P<0.001). However, there was no difference of mRNA expression of other surfactant proteins i.e. (SPB, SPC, and SPD) between postnatal day 4 and postnatal day 7 (Figure 4.8).

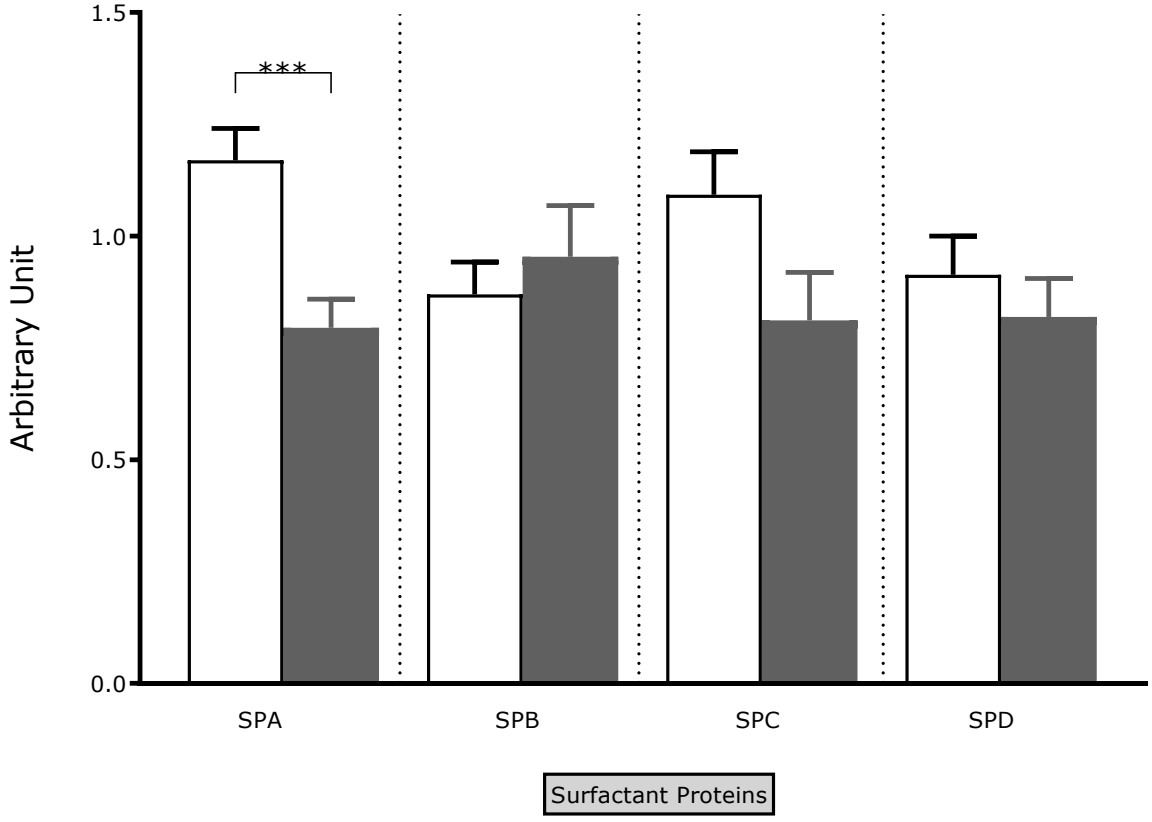


Figure 4.8 Ontological expression of mRNA surfactant proteins comparing postnatal days 4 (n=12, white bars) and 7 (n=14, grey bars). Values are mean ± standard error of mean. *** p<0.001.

The mRNA expression of MCP1 was significantly decrease between postnatal day 4 and 7 (1.1±0.1 vs. 0.7±0.1, respectively. P<0.01). In addition, the mRNA expression of TNFα showed a significant decrease between postnatal day 4 and 7 (1.0±0.08 vs. 0.7±0.1, respectively. P<0.05) as did IL1β (D4 1.2±0.1 vs. D7 0.9±0.1, P<0.05). However, the mRNA expression of the other inflammatory mediators i.e. (NFKβ, TLR4, and TGFβ1) revealed no difference between postnatal day 4 and postnatal day 7 (Figure 4.9).

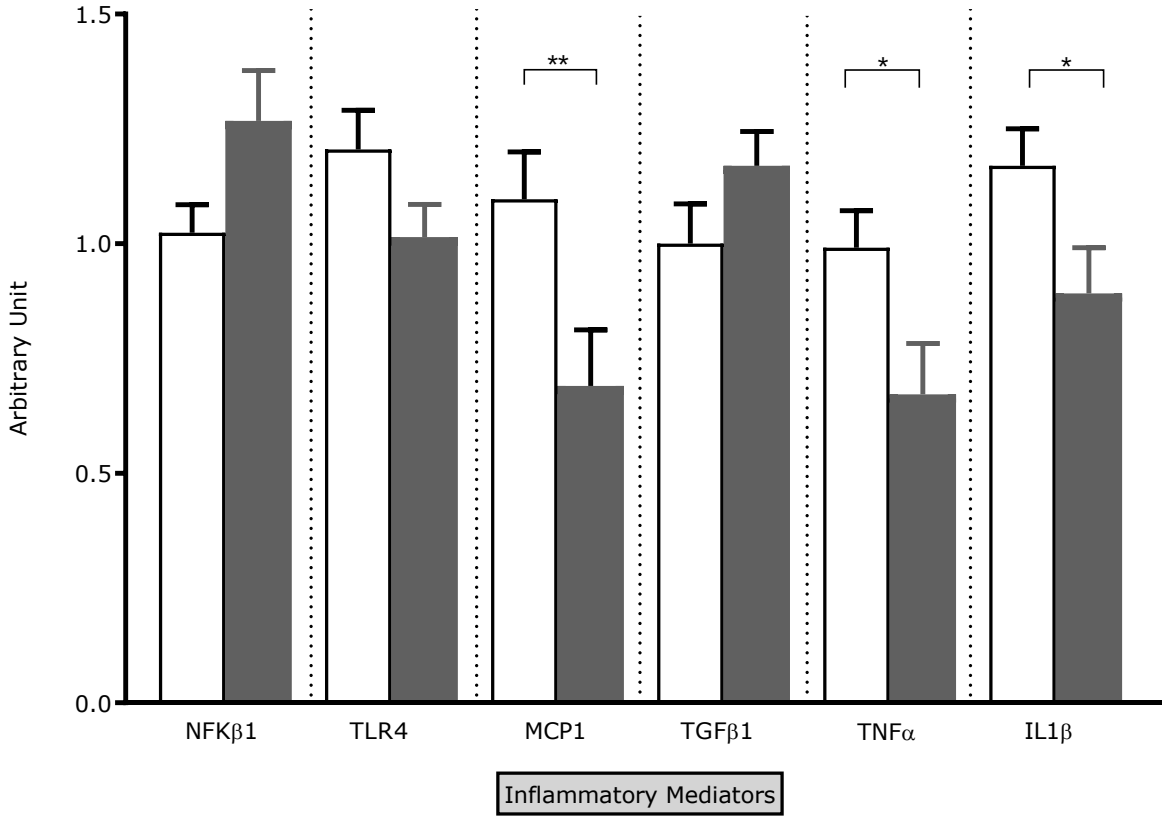


Figure 4.9 Ontological expression of mRNA inflammatory mediators comparing the postnatal day 4 (n=12, white bars) & 7 (n=14, grey bars). Values are mean ± standard error of mean. *p<0.05, **p<0.01.

4.5.2. The effect of vibration on the lung

4.5.2.1. Effect of vibration on lung mRNA expression of surfactant proteins

There were no statistically significant differences observed in the change of the mRNA expression of all surfactant proteins (i.e. SPA, SPB, SPC, and SPD) among the groups; control versus moderate or high vibration in both postnatal day 4 and postnatal day 7 as shown in Figure 4.10.

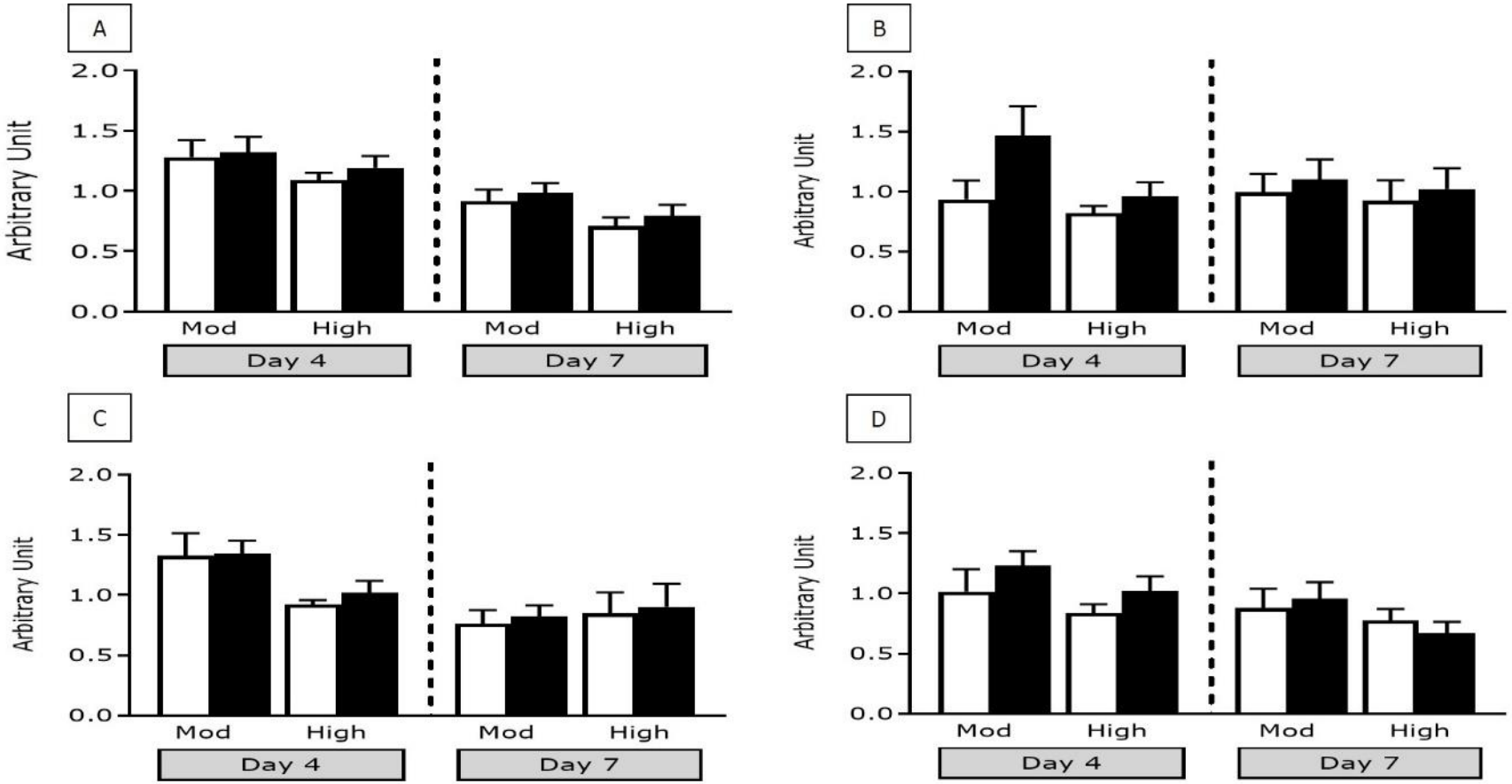


Figure 4.10 The effect of moderate and high vibration on lung mRNA expression of surfactant proteins at postnatal day 4 and 7 rat pup, white bars represent control groups and black bars represent vibration groups.

A figure for SPA expression, B figure for SPB expression, C figure for SPC expression, and D figure for SPD. Day: number of postnatal days. The mod denotes the moderate level of vibration (0.9 m/s^2), the high denote the high level of vibration (2.0 m/s^2). In the postnatal day 4: mod control group (n=5), mod vibration group (n=6), high control group (n=7), high vibration group (n=7). In the postnatal day 7: mod control group (n=6), mod vibration group (n=7), high control group (n=8), and high vibration group (n=8). The values are mean ± standard error of mean.

4.5.2.2. The effect of vibration on lung mRNA expression of NFKβ and TLR4

The mRNA expression of NFKβ and TLR4 showed no statistically differences found among groups after exposure to either level of vibration (i.e. moderate or high) compared to control groups, at both postnatal day 4 and postnatal day 7 as shown in Figure 4.11.

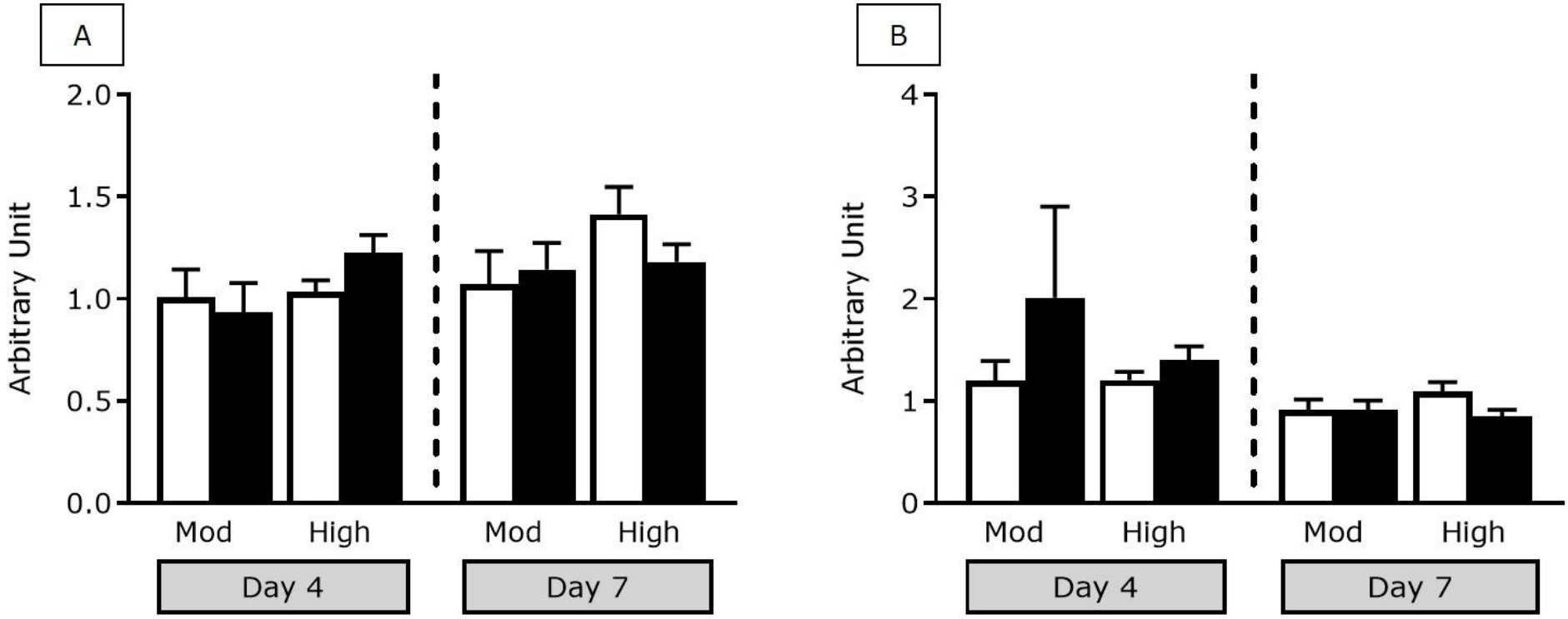


Figure 4.11 The effect of moderate and high vibration on lung mRNA expression of NFKB and TLR4 at postnatal day 4 and 7 rat. White bars represents control groups and black bars represent vibration groups.

A figure for NFKβ expression, and B figure for TLR4 expression. Day: number of postnatal days. The mod denotes the moderate level of vibration (0.9 m/s^2), the high denote the high level of vibration (2.0 m/s^2). In the postnatal day 4: mod control group (n=5), mod vibration group (n=6), high control group (n=7), high vibration group (n=7). In the postnatal day 7: mod control group (n=6), mod vibration group (n=7), high control group (n=8), and high vibration group (n=8). The values are mean ± standard error of mean.

4.5.2.3. The effect of vibration on lung mRNA expression of IL1β and TNFα

There were no statistically significant differences found in the change of mRNA expression of the proinflammatory cytokines IL1β and TNFα among the groups (i.e. controls versus moderate or high vibration) at postnatal day 4 and postnatal day 7 as shown in Figure 4.12.

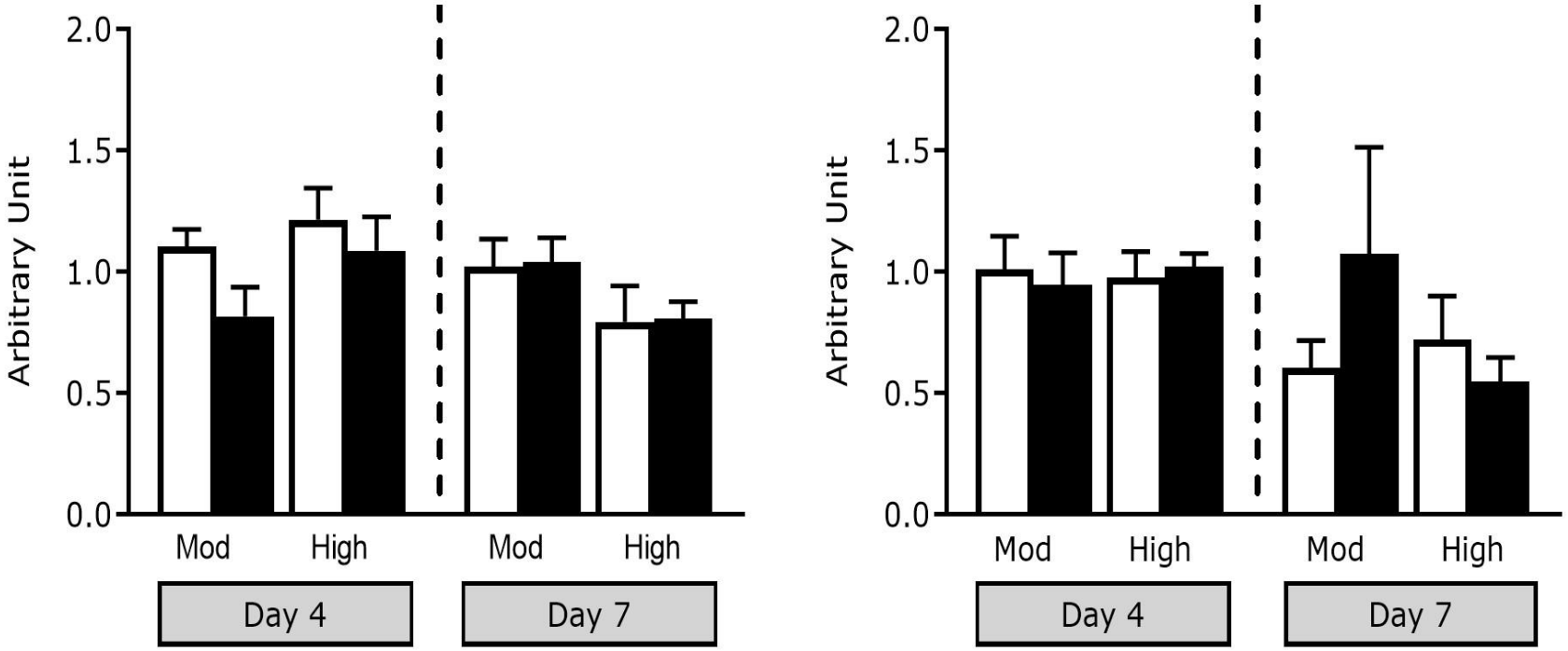


Figure 4.12 The effect of moderate and high vibration on lung mRNA expression of proinflammatory cytokines IL1β and TNFα. White bars represent control groups and black bars represent vibration groups.

A figure for IL1β expression, and B figure for TNFα expression. Day: number of postnatal days. The mod denotes the moderate level of vibration (0.9 m/s^2), the high denote the high level of vibration (2.0 m/s^2). In the postnatal day 4: mod control group (n=5), mod vibration group (n=6), high control group (n=7), high vibration group (n=7). In the postnatal day 7: mod control group (n=6), mod vibration group (n=7), high control group (n=8), and high vibration group (n=8). The values are mean ± standard error of mean.

4.5.2.4. The effect of vibration on lung mRNA expression of MCP1

The mRNA expression of the proinflammatory chemokine MCP1 showed no statistically significant difference after exposure to any level of vibration (i.e. moderate and high) at any of the postnatal age (i.e. postnatal day 4 and 7). However, the mRNA expression showed a borderline tendency of higher expression following vibration in 7 days moderate vibration groups compared to its control group (0.82±0.15 vs. 0.43±0.04 respectively, *P*=0.05) as shown in Figure 4.13.

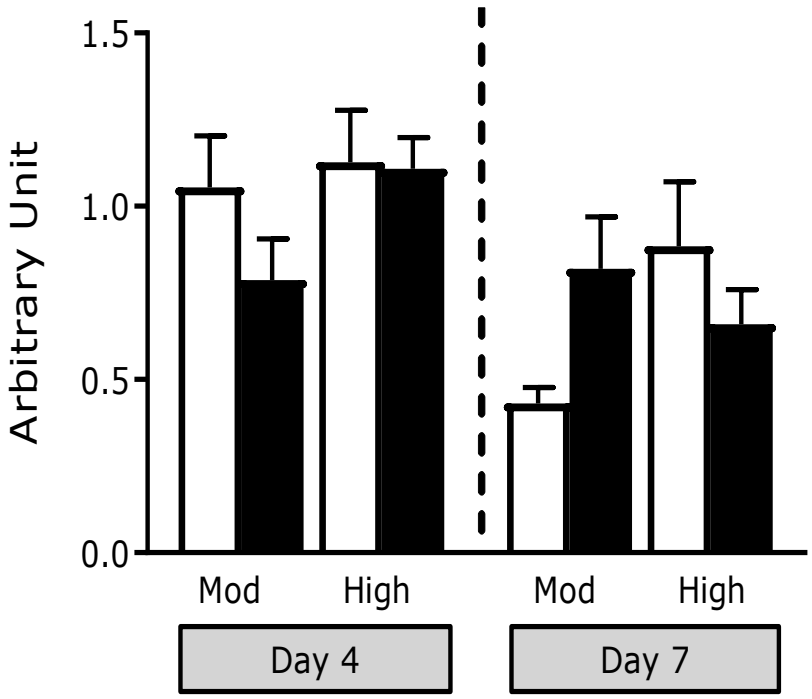


Figure 4.13 The effect of moderate/high vibration on lung mRNA expression of chemokine MCP1, white bars represent control groups and black bars represent vibration groups.

Day: number of postnatal day. The mod denotes the moderate level of vibration (0.9 m/s^2), the high denote the high level of vibration (2.0 m/s^2). In the postnatal day 4: mod control group (n=5), mod vibration group (n=6), high control group (n=7), high vibration group (n=7). In the postnatal day 7: mod control group (n=6), mod vibration group (n=7), high control group (n=8), and high vibration group (n=8). The values are mean ± standard error of mean.

4.5.2.5. The effect of vibration on lung mRNA expression of TGFβ1

There was no statistically significant difference in mRNA expression of the TGFβ1 cytokine found after exposure to any level of vibration (i.e. moderate and high) compared to control groups at any of the postnatal age (i.e. postnatal day 4 and postnatal day 7) as shown in Figure 4.14.

Summary of normalised mRNA expression comparing control with intervention groups is attached in Appendix 18.

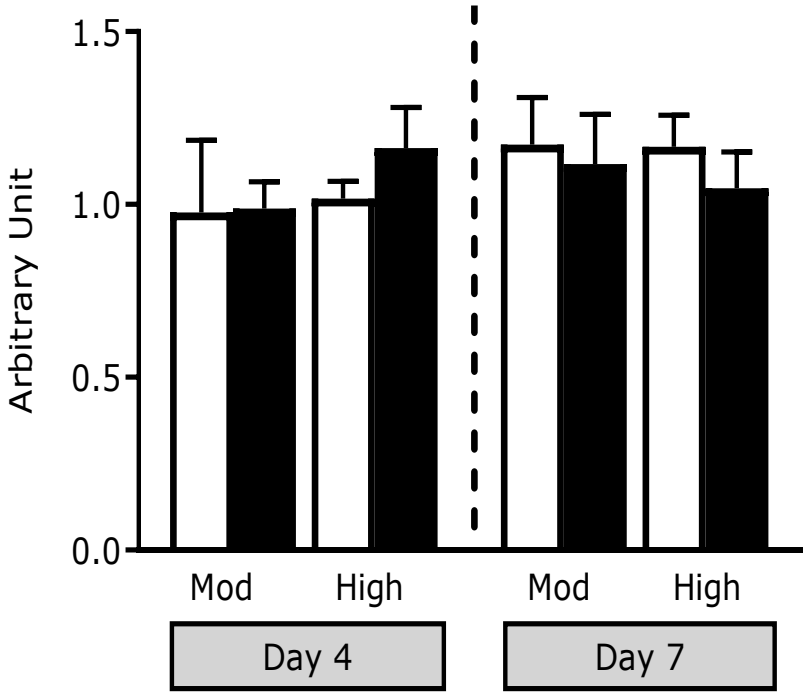


Figure 4.14 The effect of moderate/high vibration on lung mRNA expression of growth factor TGFB1, the white bars represent control group and black bars represent vibration group.

Day: number of postnatal days. The mod denotes the moderate level of vibration (0.9 m/s^2), the high denote the high level of vibration (2.0 m/s^2). In the postnatal day 4: mod control group (n=5), mod vibration group (n=6), high control group (n=7), high vibration group (n=7). In the postnatal day 7: mod control group (n=6), mod vibration group (n=7), high control group (n=8), and high vibration group (n=8). The values are mean ± standard error of mean.

4.5.2.6. The effect of vibration on lung histological structure

There were no statistically significant differences in the lung injury score observed after exposure to either moderate or high vibration compared to control groups at postnatal day 4 and postnatal day 7 as shown in Figure 4.15, off note lung injury score of 1 indicates the presence of significant injury.

The histological analysis of H&E stained lung slides demonstrated no evidence of lung injury across the groups, comparing control to either moderate or high vibration at postnatal day 4 and postnatal day 7. The recruitment and infiltration of neutrophils, which is a key marker of acute lung injury, in either alveolar or interstitial space was not observed histologically. No statistically significant difference in alveolar septal thickness, hyaline membranes, and proteinaceous debris filling the air spaces as shown in Figure 4.16.

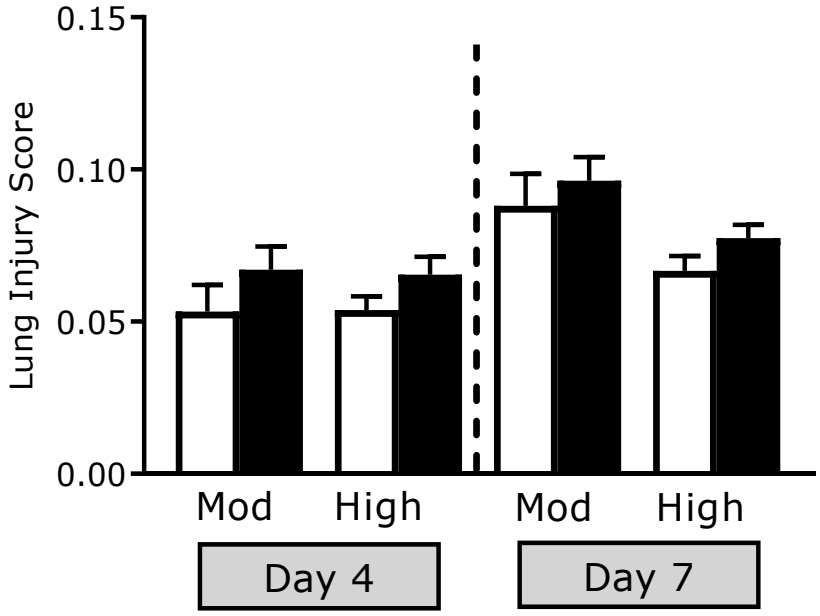


Figure 4.15 The effect of moderate/high vibration on histological lung injury scoring at postnatal age day 4 and 7

Day: number of postnatal day. The mod denote the moderate level of vibration (0.9 m/s^2), the high denote the high level of vibration (2.0 m/s^2). In the postnatal day 4: mod control group (n=5), mod vibration group (n=6), high control group (n=7), high vibration group (n=7). In the postnatal day 7: mod control group (n=6), mod vibration group (n=7), high control group (n=8), and high vibration group (n=8). The values are mean ± standard error of mean.

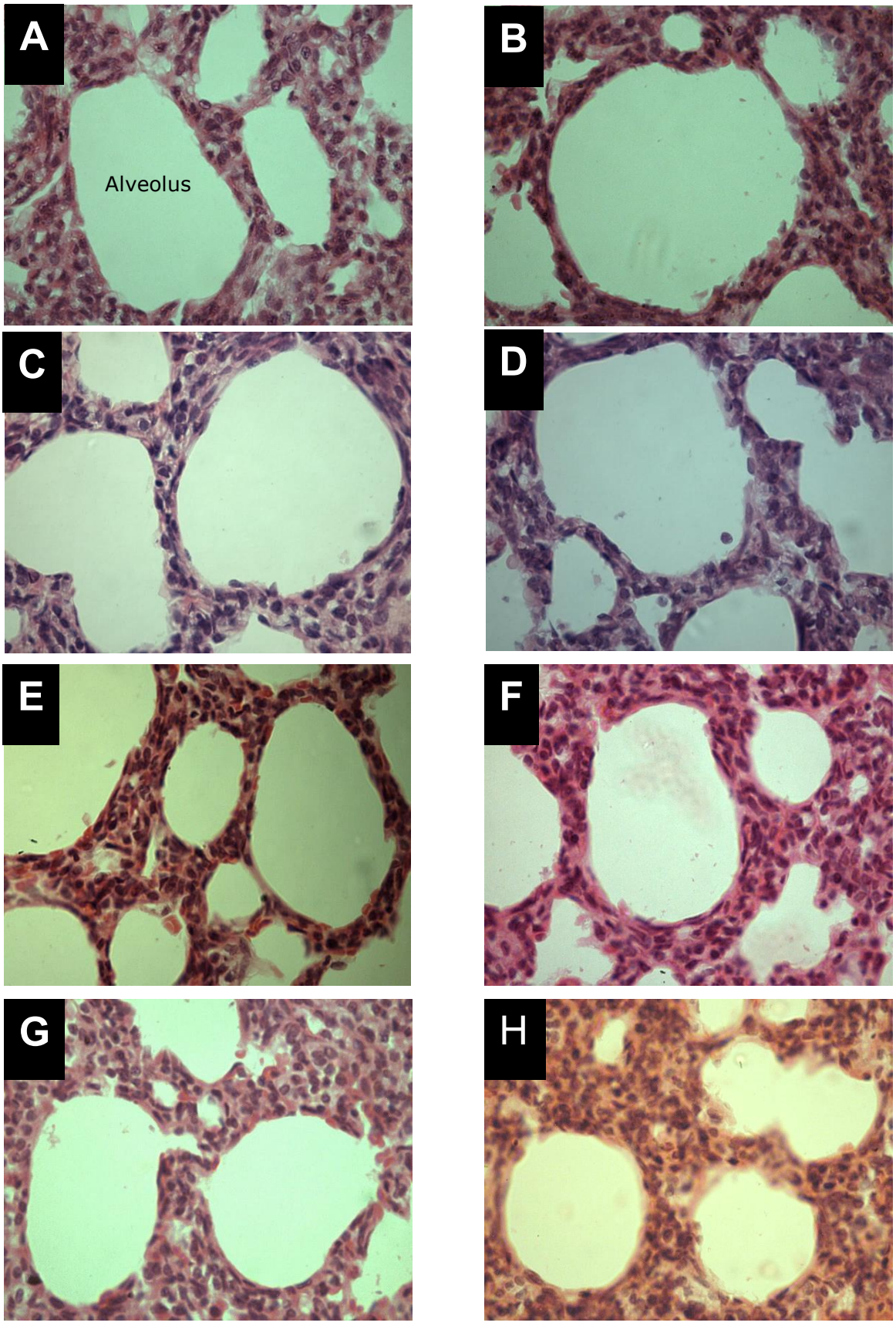


Figure 4.16 Representative histological microphotographs of lung tissue. Lung section stained with Haematoxylin and Eosin (magnification of x400. [A] postnatal day (PD) 4 moderate (mod) Control group, [B] PD4 mod Vibration group, [C] PD4 high control group, [D] PD4 high vibration group, [E] PD7 mod control group, [F] PD7 mod vibration group, [G] PD7 high control group, [H] PD7 high vibration group.

4.6. Discussion

This study was designed to quantify and assess the impact of vibration, as experienced during inter-hospital transport, on injury risk to newborn lungs using rat model. There was no significant differences between control and vibration groups in the relative gene expression of surfactant proteins and inflammatory genes at postnatal day of 4 and 7. Histological analysis found no differences between the control and vibration groups. Together, these findings suggest that WBV does not have a harmful effect on lung histological structure and inflammatory gene changes in this model.

4.6.1. The ontological expression of surfactant proteins and inflammatory mediators

The ontological expression of surfactant proteins and inflammatory mediators between the ends of saccular or beginnings of alveolarisation lung developmental stage (i.e. postnatal day 4) and early alveolarisation stage (i.e. postnatal day 7) were detailed.

In this study, the mRNA expression of SPA significantly reduced as the postnatal age advanced from day 4 to day 7. The remaining surfactant proteins (i.e. SPB, SPC, and SPD) did not show any differences between the two age points. The ontogeny expression of surfactant proteins between near term and early term rat pups has not previously reported. However, there is one study that compared the ontological expression of all surfactant proteins at postnatal day 3 and 6 in reference to newborn rats at postnatal day 1 [245]. This study compared the surfactant protein expression at late saccular and early alveolarisation, with respect to early saccular stage, and found that the expression of all surfactant proteins was high at birth and significantly reduced during lung development [245]. It is difficult to compare between the present study and this study due to the marked differences in the time points of each study.

The increased expression of SPA at postnatal day 4 compared to postnatal day 7 may be related to the role of SPA in the innate immune system as fetus exposes to acute stress during labour and delivery, and the acute stress has been shown to enhance innate immune response in early life, which subsequently reduces [246, 247]. It is therefore likely that this reflects normal physiological effects during labour and delivery.

The current study found MCP1, TNFα, and IL1β were the only inflammatory mediators with a significant reduction, as the rat pups lungs developed from the near term to term stage. The ontological expression of MCP1 demonstrated a significant reduction following high expression at postnatal day 4. The higher MCP1 in early life might have an important role in the regulation of angiogenesis, essential for early alveolarisation stage during normal lung development [248, 249].

The ontogeny of IL1β and TNFα expression in the neonatal rat lung has not previously reported. My results show that proinflammatory cytokine expression was significantly lower in lung at postnatal day 7 compared to postnatal day 4. The high expression of IL1β at postnatal day 4 might be explained by its role in lung morphogenesis during the saccular stage before a subsequent reduction during the alveolar stage [250]. While the high expression of TNFα in early life during late saccular stage might be due to its unique role in cell proliferation and differentiation during lung development [251].

TGFβ1 expression was not different between the two age points perhaps reflecting the importance of TGFβ1 signalling as key mediator for both early and late normal lung development [252].

4.6.2. WBV during inter-hospital transfer and bronchopulmonary dysplasia

The incidence of BPD has been reported to increase in postnatal transported infants compared to inborn preterm infants [69] although this was not seen in my data (chapter 3). There are many prenatal and postnatal risk factors that could increase the risk of BPD for postnatal transported infants including less antenatal steroid administration and the increased need of MV [69]. It is not clear if WBV during

inter-hospital transfer is also a factor leading to lung injury and ultimately BPD. Shah *et al* previously suggested that WBV contributed to the development of lung injury [85]. However, my study does not support WBV during transfer as risk factor for BPD.

The present study used a model that mirrored real-life inter-hospital transfer in regards of the age of the subjects and the level of vibration. The current model used postnatal day 4 and 7 rat pups, these two age points are equivalent to near term and term human respectively in regard to lung development (i.e. postnatal day 4 represents the late saccular stage and postnatal day 7 represents early alveolarisation stage) [219, 222, 223]. The amount of vibration and range of time used are the same as determined by Blaxter *et al* who quantified WBV during ambulance transportation in the UK [80]. The model studies here did not show any evidence of lung injury, based on the mRNA expression of surfactant proteins and inflammatory mediators, as well as the histological lung injury scoring between the control and two level of vibration used at two age points. However, the mRNA expression of MCP1 was of borderline significance with higher expression following moderate vibration at postnatal day 7. This could be a chance finding, as the histological assessment for the same group did not indicate the presence of injury and there was no evidence of macrophage infiltration.

To the best of my knowledge, there is only one study that explored the impact of vibration on the lung, that conducted by Shah *et al* [85]. Shah's study found that after significant WBV exposure, respiratory mechanics and lung compliance deteriorated. The findings of this study are contrary to these findings, the differences could be attributed to methodological factors. Shah's study exposed rats to a significantly higher impulse of vibration compared to the amount seen during inter-hospital transfer as determined in previous studies [75, 76, 80, 81]. Furthermore, Shah's study used positive pressure ventilation by attaching the rat pups to MV after vibration with the setting of 10ml/kg tidal volume and 30cmH_2O inspiratory pressure, these MV parameters are known to cause lung injury and

initiate an inflammatory response in rats and preterm infants [253-255]. Finally, Shah's study used rat pups at postnatal days 11 or 12. The rodent lung at these stages has undergone late alveolarisation stages and is equivalent to almost 1 year of infant life in regard to lung development [222]. Therefore, using Shah's study findings to describe the impact of vibration on neonatal settings may not be truly translatable.

My findings agree with previous animal studies that reported the use of direct vibration of less than 10 m/s^2 does not cause lung injury. However, it does improve minute ventilation and supports normal physiological breathing during conventional ventilation [256, 257]. Moreover, previous animal studies have described the use of high-frequency oscillatory ventilation (HFOV), a type of ventilation that uses vibration to move the air into the lung in the neonatal setting. The use of HFOV is safe and can reduce the incidence of lung injury, and is considered to be a protective way to ventilate injured lungs in critical care settings [258-261]. Vibration is commonly used to manage and improve respiratory health in neonatal settings. The use of vibration chest physiotherapy is one of the treatment modalities in the NICU to clear lung secretions and this appears to have no adverse outcomes on the respiratory system [262].

Although this model appears not to cause lung injury it was noted that the same experiment induced significant brain injury as reported by my colleague Dr Lara Shipley. Dr Shipley's findings support previous literature that observed an increase in the incidence of IVH among postnatal transported infants compared to none transported infants [73, 263].

4.7. Study strengths and limitations

4.7.1. Study Strengths

There are several important areas where this study may contribute to furthering our understanding of neonatal inter-hospital transport. The study method investigates the level of vibration equivalent to that experienced during real-life

ambulance transportation. The new model using different levels of WBV, this could be useful for exploring dose-dependent effects. In this model, both moderate and high WBV resulted in brain but not lung injury, this differential organ effect aligns well with preterm infant pathology. By isolating WBV from other lung injury mechanisms, such as the use of oxygen and MV, the model allowed exploration of vibration only. In addition, postnatal day 4 and 7 in the rat are equivalent to near term and early term in human lung development [222, 223]. Therefore, these two age points are valuable to represent the impact of vibration in early-life lung development.

4.7.2. Study limitations

There are some limitations of this study. The scale of the vibration in the model was defined using human neonatal patient and preterm manikin data. It is unclear how to scale WBV from that which causes adult human injury, to that seen in newborn infants and then down to the size of newborn rat pup. The vibration level is likely to be significant for the rat pup but it did not induce lung injury when delivered in the range of that seen during newborn transport. In addition to the limitations, the translation of animal model findings into humans is not always directly demonstrated and so caution is needed when trying to extrapolate this.

No power calculation was conducted for the current study as it was the first time this model had been used to explore the effect of WBV on tissue injury. Therefore, there was no data to base a power calculation on. Finally, there was unequal distribution of offspring by gender; and this lead to an imbalance between the number of males and females among the groups.

4.8. Conclusions

In conclusion, rat pups at postnatal day 4 and 7 exposed to two levels of WBV, as experienced during neonatal inter-hospital ambulance transport, did not reveal any difference of mRNA expression of surfactant proteins and inflammatory mediators. Moreover, the histological analysis did not show any evidence of lung injury.

These findings do not support the hypothesis that WBV alone contributes to the increased incidence of BPD reported among postnatal transported infants. There are other factors during transport that could contribute to increase BPD incidence such as the use of more MV and oxygen therapy.

4.9. Future work

It is not possible to attribute the increase of BPD incidence to the exposure of vibration during inter-hospital transport. Therefore, in order to investigate whether inter-hospital transport conditions associated with BPD development, it would be ideal to conduct further investigations using MV with the same vibration parameters, the parameters of MV should be within the range of real transportation including the amount of oxygen used. Furthermore, it would be useful to measure respiratory mechanics and physiological measurements, such as airway resistance and dynamic compliance while using MV. It is possible that the activation of lung inflammation and injury may occur with invasive ventilation and oxygen therapy, any additive effect of WBV then could be more apparent. However, this methodology is technically challenging in rat pups.

Chapter 5. Targeted oxygen saturation compliance in preterm infants: Application of evidence through a local quality improvement project

5.1. Overview

There are around 60,000 preterm infants born every year in the UK [6]. Preterm infants, especially very and extremely preterm infants, are born during the late canalicular and early saccular lung developmental stage. This disturbs the normal anatomical and physiological development of the lungs [20, 21] resulting in many needing respiratory support.

Oxygen is the most commonly used therapy in neonatal settings, alone or integral with other support modalities [264]. Preterm infants are at risk of hyperoxaemia, defined as SpO_2 >95% while on oxygen therapy, increasing the risk of BPD and ROP [94]. Moreover, spending more time with SpO_2 <91% can increase mortality and NEC [94]. It is recommended that SpO_2 is targeted between 91 and 95% but this can be difficult to achieve [265]. The success of SpO_2 targeting depends on many factors; including alarm limits settings, HCPs knowledge, and clinical stability of preterm infants.

Two previous studies reported the use of a Quality improvement (QI) approach to improve SpO_2 targeting and avoid hyperoxaemia. Ford *et al* found that the use of a PDSA approach to implement new FiO_2 titration guidelines, and educate HCPs, was successful in increasing the percentage of time within the target range and decreasing time above target range [266]. Lau *et al* found that the PDSA approach, as implemented by Ford, decreased time above target range [267]. Both projects illustrate the ability of the PDSA approach to change clinical practice and minimise the time SpO_2 was outside the target range. However, they have two main drawbacks: they used rapid cycle where they report the finding of combined interventions (i.e. education and guideline), and they did not report objective measurements in either compliance of setting alarm limits, and impact of

educational sessions. Therefore, in my QI project I aimed to overcome these drawbacks.

The project aimed to explore the potential of a QI project, combining three individual components, to improve SpO_2 targeting. These were: 1) educating HCPs to increase the awareness of SpO_2 targeting and hyper/hypoxaemia hazards, 2) provide the NICU clinical teams with performance data on the percentage of time in the target oxygen saturation range, and 3) narrow the SpO_2 alarm limits. These could improve the clinical team practice/behaviour and hence compliance to maximise the time in the target range and therefore improve clinical outcomes including a reduction in BPD.

I developed the NeoSat QI project. This involved additional training for NICU HCPs about the importance of SpO_2 targeting, a rapid audit and feedback of the compliance to set SpO_2 alarm limits correctly, and new SpO_2 alarm limits.

5.2. Aim and Hypothesis

5.2.1. Aim

The NeoSat QI project aimed to increase the time preterm infants on oxygen therapy spent within the recommended SpO_2 target range, i.e. SpO_2 of 91%-95% [130].

5.2.2. Hypothesis

A QI approach that improves HCPs knowledge regarding the SpO_2 targeting range, combined with narrower SpO_2 alarm limits, could increase the time SpO_2 is kept in the recommended target range.

5.3. Methods

5.3.1. Use of Quality improvement project

In healthcare settings, a QI project is a systematic framework model used to improve the quality of delivered care [268]. One of the common QI methods used to improve healthcare delivery is the Plan-Do-Study-Act (PDSA) cycle described in Table 5.1 [269]. The PDSA approach depends on assessing whether a particular intervention could achieve the desired improvement and allow for modification to ultimately achieve the desired aims [270].

Table 5.1 Description of plan-do-study-act approach used to assess and improve healthcare services [269].

Item	Description
Plan	Define the gap in neonatal healthcare practice, identify the aim, and formatting the hypothesis for improvement
Do	Conduct data collection to find the baseline data in the first cycle and analyse the improvement after changes in subsequent cycles
Study	Analyse and interpret the findings, summarise what was learnt, and what was wrong
Act	Adopt the change or reject it and plan the next cycle for more improvement

In contrast to clinical trials, the PDSA approach allows for method revision and a new learning intervention to be built into the experiment. Subsequent PDSA cycles are conducted to see if the problem has been resolved or further challenges need to be addressed [270].

The PDSA cycle method was previously used in neonatal settings and found to be effective in guideline implementation, service evaluation, and overall to facilitate continual improvement [271, 272].

5.3.2. Design

NeoSat was a prospective, observational QI project focusing on preterm infants at risk of BPD. The team composed of a consultant neonatologist Dr Don Sharkey, the nursing educators of both NICUs, and myself as the investigator.

5.3.3. Setting

The NeoSat project was conducted in two NICUs at Nottingham University Hospitals NHS Trust in Nottingham, UK, during the period from August 2018 to September 2019. These NICUs are level 3 tertiary centres caring for all preterm infants. Both centres monitor and measure SpO_2 continuously by pulse oximetry. The local guidelines, for preterm infants <33 gestational age on oxygen therapy, defines the target range to be 91% - 95%; this is aligned with BOOST II trial [130], and SpO_2 alarm limits are set at 80% - 96% whilst in oxygen.

5.3.4. Sample

The NeoSat QI project includes two groups of interest, preterm infants and HCPs. The inclusion criteria for the preterm infants consisted of premature infants born with gestational age less than 33 weeks (i.e. very and extremely preterm infants), receiving respiratory support (MV and non-invasive ventilation) and with parental verbal consent. Exclusion criteria were infants with major congenital malformations or with a different SpO_2 target range to the normal guideline (e.g. congenital heart disease or pulmonary hypertension). A convenience sample was used for infants and the healthcare team participation was voluntary.

5.3.5. Interventions

The NeoSat project used a structured QI method with three consecutive PDSA cycles: assess the problem during baseline measurement, perform HCPs assessment, develop the educational package, perform educational sessions, change the lower SpO_2 alarm limit, and collect data to evaluate the effectiveness of interventions.

For each cycle, Assessment of HCP knowledge's, percentage of time SpO_2 kept in optimal oxygen target range, and compliance of setting SpO_2 alarm limits correctly were collected. All three PDSA cycles timeline and interventions summarised below in Table 5.2.

Table 5.2 Summary of the NeoSat cycles, illustrating the duration and the interventions for each cycle.

Cycle of NeoSat	Duration	Intervention
1st PDSA cycle	Aug 2018 to Dec 2018	Baseline data collection of oxygen targeting and alarm limits
	Jan 2019 to Mar 2019	Baseline measurement of HCPs knowledge
2nd PDSA cycle	April 2019 to Mid-June 2019	Feedback baseline findings and conduct educational sessions
	Mid-Jun 2019 to mid-Jul 2019	Data collection of oxygen targeting and alarm limits
3rd PDSA cycle	Mid-Jul 2019 to end of Jul 2019	Feedback 2nd cycle findings and change SpO_2 lower alarm limit
	Aug 2019 to Sep 2019	Data collection of oxygen targeting and alarm limits
PDSA: plan- do-Study- act; HCPs: healthcare professionals; SpO_2: peripheral capillary oxygen saturation.		

5.3.5.1. First PDSA cycle

The first PDSA cycle aimed to establish the baseline data for the units for the following three elements: 1) targeting the appropriate SpO_2 range, 2) the baseline compliance of HCPs to set the SpO_2 alarm limits correctly, and 3) assess HCPs knowledge regarding SpO_2 targeting and hazards of both hyperoxaemia and hypoxaemia.

Arm 1: Measurement of baseline for targeting appropriate SpO_2

After parental permission was verbally agreed, the SpO_2 data were collected using the oxygen graphical user interface (OxyGUI) software system. The OxyGUI system connected to the patient monitor and oxygen analyser. Included infants were given a unique ID number, and infant data including gestational age and corrected gestational age were recorded.

OxyGUI software

OxyGUI is a bespoke password protected data collection software developed by the Department of Child Health in collaboration with engineers at the University of Nottingham. It stores raw data including the date, time, SpO_2, HR, FiO_2 and signal quality. This software automatically captures the data at one-second intervals as outputted by the main clinical monitor. The OxyGUI data are recorded as comma-separated values using Microsoft Excel (Microsoft Corporation). If the system interrupted (e.g. displacement of pulse oximetry electrode), the software records a signal quality value of zero.

OxyGUI System set up

A laptop (Lenovo ThinkPad, Basingstoke UK) with the OxyGUI software was connected to the infant's monitor (GE CARESCAPE monitor B650, General Electric Company) (Figure 5.1). The oxygen analyser AX300 (Viamed, UK) was calibrated to room air for as per the manufacturer's instructions then placed in the inspiratory limb of the infant's breathing circuit. The oxygen analyser then outputs the analogue data to a National Instruments data acquisition unit (National

Instruments DAQ USB6008, Newbury UK) which sends the analyser data to the software. All the data is stored on the laptop (no patient identifiable data) and is collected offline later. There were four systems used for this project to allow multiple recordings to be made.

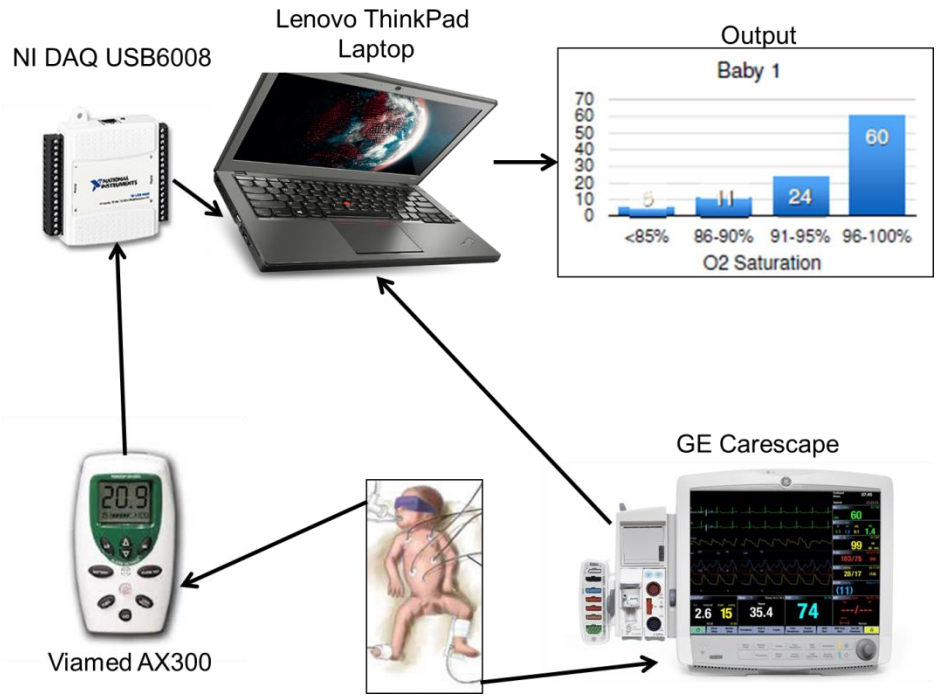


Figure 5.1 OxyGUI system set up

The laptop and connecting cables were securely placed in the suitable shelf behind the patient monitor. The safety check for all systems was conducted by the Trust's clinical engineering team before first use. All equipment was used as per the manufacture's recommendations and only audit team members operated the system.

Arm 2: HCP compliance with SpO_2 alarm limits settings

For preterm infants born with gestational age less than 33 weeks and in oxygen, the local NICU guideline recommended that the upper SpO_2 alarm limit should be set at 96% and lower SpO_2 alarm limit at 80%. However, the lower SpO_2 alarm limit changed on the third PDSA cycle from 80% to 88%. The 80% lower limit was

considered correct in the first and second cycles, 88% was considered correct in the third cycle only. The set SpO_2 upper and lower alarm limits for preterm infants were collected every day and always at least three hours after the commencement of a day shift until the end of the data collection period for each cycle. SpO_2 data and alarm limits were collected from August 2018 until December 2018. Project data were completed manually using the data collection form as shown in Figure 5.2.

SPO2 Targeting Range (Phase I)

Baseline Data

	Date:			**NICU Unit:**	QMC ☐	City Hospital ☐	
	SPO_2 Alarm Limit						
Baby #	Bay/Bed	Baby Gestational Age	Corrected Gestational Age	SPO_2 Upper Alarm Limit	SPO_2 Lower Alarm limit	Oxygen Y : Yes N: No	Cyanotic Heart Disease Y : Yes N: No
1							
2							
3							

Figure 5.2 Sample of SpO_2 alarm limits collection form used during first cycle

Arm 3: Assessment of HCPs knowledge regarding oxygen targeting

HCPs knowledge was measured using a web-based survey exploring the practice of targeting SpO_2 in both local NICUs. The questions consisted of three main sections:

- *Section 1:* Understanding of what is the appropriate SpO_2 targeting range
- *Section 2:* Assess knowledge on the correct SpO_2 alarm limits for preterm infants
- *Section 3:* Assess knowledge of conditions associated with periods outside the desired target saturation range

The survey was initially piloted and sent to a small number of HCPs for feedback. Responses with improvement recommendations were obtained. The final version of

the survey (Appendix 19) was used to measure baseline knowledge (pre-intervention) and improvement (post-intervention) following a period of education. Surveys were designed and conducted using Jisc Online Surveys (Jisc, Bristol, UK). The completion of the survey was voluntary, and consent was implied by completion of the survey. The pre survey was sent out during the first PDSA cycle to all NICUs HCPs via email. Two reminder emails were sent at 4-week intervals and the survey closed 2 weeks after.

5.3.5.2. Second PDSA cycle

The second PDSA cycle aimed to improve both SpO_2 targeting and compliance to set SpO_2 alarm limits after feedback of the baseline results and educational sessions to the HCPs.

The education package (Appendix 20) was delivered over 8 weeks (April 2019 to mid-June 2019). The package consisted of providing all of the healthcare team with baseline data, emphasising the importance of targeting SpO_2 and highlighting the importance of setting SpO_2 alarm limits correctly.

The educational package was shared using a variety of methods including: an email to all of the healthcare team; educational sessions conducted for staff nurses every day by the unit nurse educator before the morning shift started; and delivery to medical staff by a neonatal consultant (Dr Sharkey) during teaching sessions. As part of the educational package, a visual reminder was placed on every infants' monitor and around staff communal area information boards to remind the healthcare team of SpO_2 targeting range (Figure 5.3).

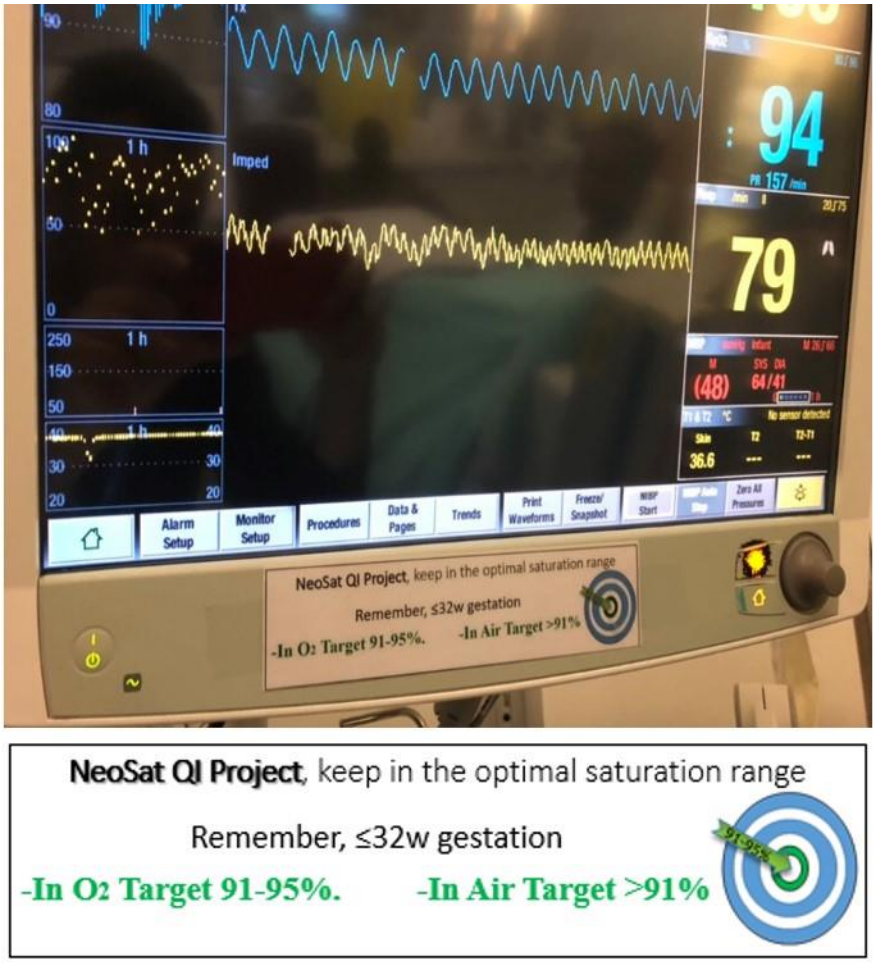


Figure 5.3 Visual reminder note of SpO_2 targeting for preterm infants less than 33 gestational age placed on all NICU monitors during the second cycle

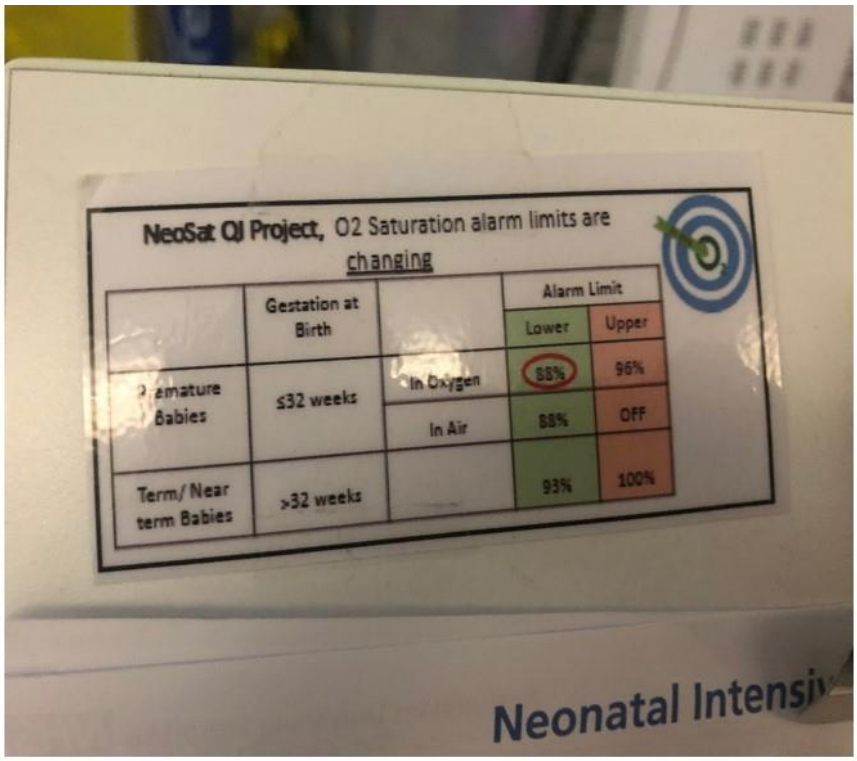
After the educational phase, the second round of observations were conducted to measure the percentage of time HCPs achieved the target SpO_2 and the compliance with setting alarm limits correctly (mid of June 2019 to mid of July 2019).

5.3.5.3. Third PDSA cycle

The third PDSA cycle aimed to improve both the percentage of time targeting appropriate SpO_2 was achieved and the compliance with setting SpO_2 alarm limits correctly after changing the lower SpO_2 alarm limit from 80% to 88%.

This cycle started on mid of July 2019 after the senior neonatal intensive care team agreed to my recommendation to change the lower SpO_2 alarm limit and narrow the alarm window to be 88% for the lower limit and 96% for the upper limit. All

healthcare team members were informed of this change and updated visual reminding notes were placed on every clipboard next to the nursing charts at each patient cot (Figure 5.4).



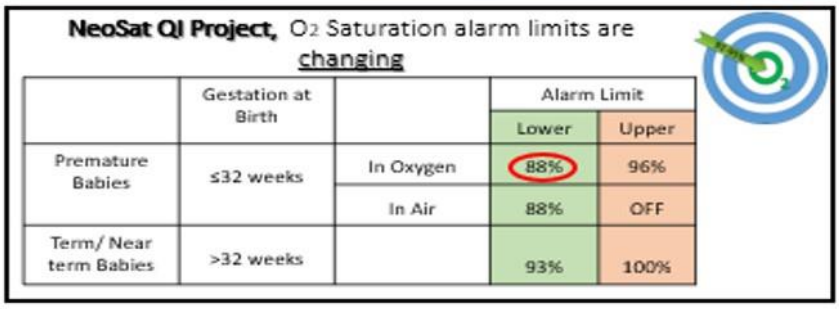


Figure 5.4 Visual reminders placed on every nurse clipboard and illustrating the new SpO_2 lower alarm limit during third cycle

After changing the lower alarm limit, an online post-survey containing the same questions included in the pre-survey, except for Likert scale assessment of difficulties to targeting SpO_2, was disseminated to all healthcare team members. The post-survey was run during August 2019, and a reminder email was sent. One of the aims of this round of the survey was to make sure that the message of changing SpO_2 lower limit had been delivered.

At the beginning of September 2019, I undertook the third measurement round of targeted SpO_2 and compliance with setting the alarm limits correctly.

5.3.6. Ethical considerations

The NeoSat project was approved as a clinical audit and QI project and so did not require ethical approval.

5.3.7. Data analysis

5.3.7.1. SpO_2 Saturation

The SpO_2 data were cleaned and organised to remove all duplicated measurements that occurred during OxyGUI software freezes and all measurements while the infant was disconnected from the monitor. When infants were in oxygen, the time in seconds within the SpO_2 ranges were collected for each infant individually and aggregated as a percentage of the recorded time.

Quantitative data are presented as mean (SD), median (IQR), or number (%) were appropriate. The D'Agostino-Pearson normality test was used to determine if data were normally distributed or not. Statistical analysis used a one-way ANOVA test for normally distributed data and non-parametric Kruskal-Wallis test for non-normally distributed data. The comparison was made to compare time below, within, and above target range throughout the QI project. All statistical analysis and were performed using GraphPad prism V 8 (GraphPad Software, California, USA).

The proportion of time spent at different SpO_2 levels for each infant were obtained, and pooled SpO_2 histogram created for each cycle using Microsoft office Excel software 2016 (Microsoft Corporation, Berkshire, UK). A master proportion table was then created by averaging the proportion at each SpO_2 level.

5.3.7.2. SpO_2 alarm limits

After the alarm limit audit, SpO_2 alarm limits were specified as correct or incorrect. Incorrect categorisation included either one or both limits were wrong. Compliance

was defined as the percentage of setting both limits correctly. Statistical analysis used the Chi-square test or Fisher-exact test to explore the relationship between setting alarm limits correctly across QI cycles, the Fisher-exact test used when small sample of less than 5 and Chi-square for larger samples. Statistical comparison was conducted using SPSS software V24 (IBM, Portsmouth UK).

5.3.7.3. Healthcare team knowledge: Pre and post surveys

Pre and post-data were imported as Microsoft Excel files and then converted to STATA file v16 (STATA) for statistical analysis. Results of the pre and post-surveys were compared for each question, and coded to be analysed as yes/no answer (e.g. for the upper SpO_2 target range question, the frequency of participants selecting the correct answer will be the yes frequency, and the frequency of all other wrong selection will be the no frequencies). The Chi-square test or Fisher exact tests used for the statistical significance different between pre and post results. For all the statistical comparison tests a P value of <0.05 determined the significant difference.

5.4. Results

5.4.1. Assessment of HCPs knowledge

5.4.1.1. Characteristics of participants

The pre-survey responses were received from 52 HCPs in both units, and post-survey responses from 39; of which 22 (56%) had participated in the pre survey. Characteristics of HCPs who participated are shown in Table 5.3.

Table 5.3 Characteristics of healthcare professionals participating in pre/post-survey that aimed to assess knowledge regarding SpO_2 targeting

Characteristics		Pre-survey (n=52)	Post-survey (n=39)
Speciality	Doctor	7 (13)	6 (15)
	ANNP	1 (2)	1 (3)
	Nurse	44 (85)	32 (82)
Number of years of neonatal experience	Less than 1	4 (8)	2 (5)
	1-3	7 (13)	8 (20.5)
	4-8	13 (25)	8 (20.5)
	More than 8	28 (54)	21 (54)
Knowing unit has guideline for SpO_2 targeting	Yes	50 (96)	32 (82)
	No	1 (2)	2 (5)
	Not sure	1 (2)	5 (13)
ANNP: advanced neonatal nurse practitioner Data presented as n (%)			

5.4.1.2. Challenges targeting appropriate SpO_2

In the first PDSA cycle, HCPs were asked to grade their thoughts regarding potential causes of why it can be hard to target SpO_2 in preterm infants. 1-100 stacked bars of the results were plotted (Figure 5.5). The instability of preterm infants and the broad SpO_2 alarm limits are the most common challenges to keep SpO_2 within the target range. Based on these findings, narrowing the alarm limits was considered during the next PDSA cycles.

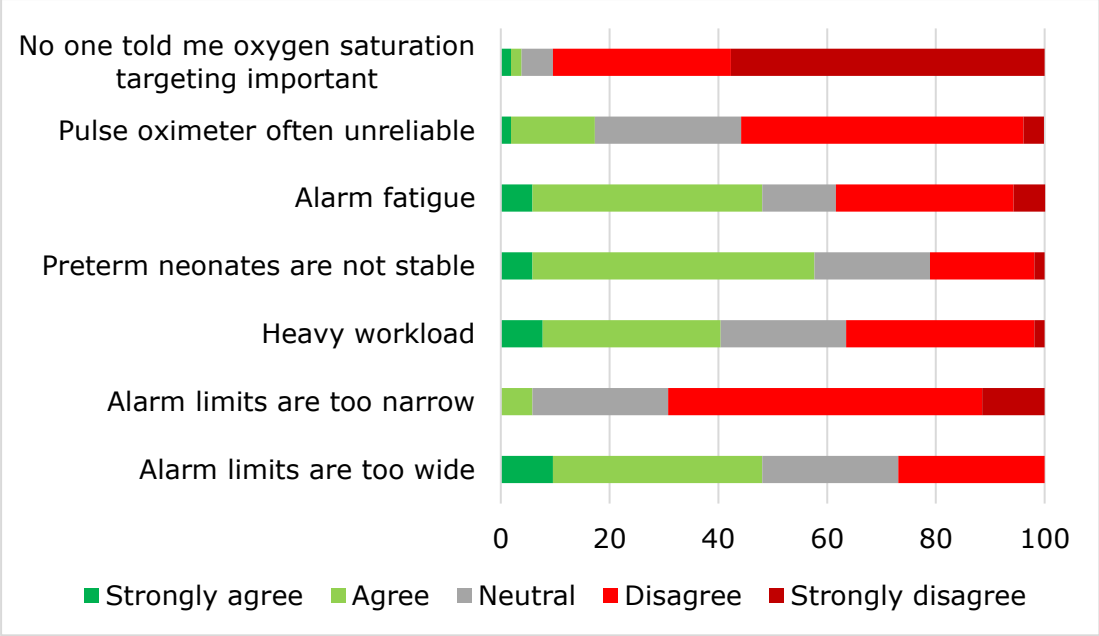


Figure 5.5 Healthcare professionals pre-education responses to the causes that could affect oxygen saturation targeting in preterm infants, n=52.

5.4.1.3. HCPs knowledge regarding SpO_2 target range and alarm limits

HCPs knowledge of the appropriate upper and lower SpO_2 targeting range did not significantly differ before or after the educational period (Table 5.4).

Table 5.4 Healthcare professionals knowledge of SpO_2 target range and alarm limits for infants <33 weeks of gestational age on oxygen therapy

Findings	Pre-education (n=52)	Post-education (n=39)	P value
Identify the correct SpO_2 upper target range	24 (46)	19 (49)	0.80
Identify the correct SpO_2 lower target range	26 (50)	22 (56)	0.54
Identify the correct SpO_2 upper alarm limit	41 (79)	34 (87)	0.30
Identify the correct SpO_2 lower alarm limit	41 (79)	34 (87)	0.30
SpO_2: peripheral capillary oxygen saturation. Data presented as n (%). Statistical comparisons completed using chi-squared test.			

5.4.1.4. HCPs knowledge regarding important morbidity associated with SpO_2 outside the target range

As can be seen by the frequencies cross-tabulated in Table 5.5, there is no significant difference between pre/post survey in regard to HCPs knowledge in identifying important morbidity, associated with either below or above SpO_2 target range. However, there was a significant difference observed between with an improvement of HCPs who identified death as being associated with keeping SpO_2 below target range (62% in pre-survey vs 85% in post-survey, p=0.02).

Table 5.5 Healthcare professionals' knowledge of the serious conditions associated with SpO_2 outside the target range

Conditions	Pre-education (n=52)	Post-education (n=39)	P value
Conditions associated with SpO_2 **above** target range			
BPD	16 (31)	14 (36)	0.61[x]
Death	5 (10)	2 (5)	0.70[f]
IVH	10 (19)	8 (20.5)	0.88[x]
NEC	5 (10)	1 (3)	0.23[f]
Late onset infection	4 (8)	2 (5)	0.70[f]
ROP	49 (94)	38 (97)	0.63[x]
Conditions associated with SpO_2 **below** target range			
BPD	36 (69)	22 (56)	0.21[x]
Death	32 (62)	33 (85)	0.02[x]
IVH	41 (79)	24 (62)	0.07[x]
NEC	41 (79)	35 (90)	0.25[x]
Late onset infection	22 (42)	12 (31)	0.26[x]
ROP	1 (2)	0 (0)	-

Data presents as n (%)
BPD: bronchopulmonary dysplasia; IVH: intraventricular haemorrhage; NEC: necrotising enterocolitis; ROP: retinopathy of prematurity
[x] Chi-squared test for sample of ≥5,[f] Fisher's Exact test for sample of <5.

5.4.2. Compliance with setting SpO_2 alarm limits correctly

5.4.2.1. Setting SpO_2 alarm limits for all preterm infants <33 weeks gestational age

In total, 1664 SpO_2 alarm limits for preterm infants were collected. These were split as 687 during the first cycle, 391 during the second cycle, and 586 during the third cycle. The frequencies and percentages demonstrate a significant relationship between the QI project cycles and setting SpO_2 alarm limits correctly with an improvement through the cycles (Table 5.6).

Table 5.6 Compliance of setting SpO_2 alarm limits for preterm infants < 33 weeks gestational age on either room air or oxygen therapy during all 3 PDSA cycles

SpO_2 alarm limits	1st PDSA cycle (n= 687)	2nd PDSA cycle (n= 391)	3rd PDSA cycle (n=586)	P value
Correct setting of both	439 (64)	327 (84)	518 (88)	<0.001[x]
Incorrect setting of both	35 (5)	4 (1)	8 (1)	<0.001[f]
Upper limit incorrect	138 (20)	21 (5)	38 (6)	<0.001[x]
Lower limit incorrect	75 (11)	39 (10)	22 (4)	<0.001[x]

Data presents as n (%)
[x] Chi-squared test for statistical comparisons
[f] Fisher-exact test for statistical comparisons

5.4.2.2. Setting SpO_2 alarm limits for preterm infants <33 weeks gestational age while on oxygen therapy

Oxygen toxicity is important in preterm infants so the compliance was explored when only in oxygen. 934 SpO_2 alarm limits were collected, 397 during the first cycle, 183 the second, and 354 during the third cycle. There was a statistically significant relationship between project cycles and correct/incorrect setting of SpO_2 alarm limits (Table 5.7).

Table 5.7 Compliance of setting SpO_2 alarm limits for preterm infants <33 weeks gestational age on oxygen therapy during all 3 PDSA cycles

SpO_2 alarm limits	1st PDSA Cycle (n=397)	2nd PDSA cycle (n=183)	3rd PDSA cycle (n=354)	P value
Correct setting of both	221 (56)	154 (84)	310 (88)	<0.001[x]
incorrect setting of both	21 (5)	2 (1)	6 (2)	<0.01[f]
Upper limit incorrect	129 (32)	19 (10)	34 (10)	<0.001[x]
Lower limit incorrect	26 (7)	8 (4)	4 (1)	<0.001[f]

Data presents as n (%)
[x] Chi-squared test for statistical comparisons
[f] Fisher-exact test for statistical comparisons

5.4.3. SpO_2 targeting

Data were collected on 37 preterm infants in this QI project, 17 infants during the first PDSA cycle (i.e. baseline measurements), 9 infants during the second PDSA cycle (i.e. after educational sessions), and 11 infants during third PDSA cycle (i.e. after narrowing the SpO_2 alarm limits). Approximately 3,000 hours were analysed for baseline data, 2,300 hours for the second cycle, and 2,200 hours for the third cycle (Table 5.8).

Table 5.8 Preterm infants gestational age and duration of recorded hours for each PDSA cycle

Characteristics	1st PDSA cycle (n=17)	2nd PDSA cycle (n=10)	3rd PDSA cycle (n=11)
Gestational age, mean (SD) weeks	27.3 (1.86)	26.6 (2.20)	25.6 (1.36)
Recorded hours per infant, median (IQR)	92 (32-158.5)	198 (120-415.5)	190 (142-240)
SD: standard deviation; IQR: interquartile range			

5.4.3.1. Percentage of time SpO_2 within, below and above appropriate target range

As the QI project progressed, preterm infants spent more time in the appropriate target range (i.e. 91-95%) compared to baseline (P=0.03) (Table **5.9**). Dunn's multiple comparison test was performed for the three cycles; there was a statistically significant difference between the baseline measurements and the last cycle but not between other cycles (i.e. first cycle vs. second cycle and second cycle vs. third cycle).

The median percentage of time spent below target range (<91%) revealed no statistically significant differences between the three cycles (Table 9).The median percentage of time spent above the target range (≥96%) did not significantly change between the three cycles (Table 5.9).

Table 5.9 Percentage of time in different SpO_2 ranges as measured during each cycle

SpO_2	1st PDSA cycle (n=17)	2nd PDSA cycle (n=9)	3rd PDSA cycle (n=11)	P value
Below target range (SpO_2 <91)	33 [21.4-48.1]	40 [15.3-42.5]	26.5 [23-29.1]	0.36
Within target range (SpO_2 91-95%)	34 [29.8–39.2]	39 [34.8–48.4]	40 [36.2–54.3]	0.03
Above target range (SpO_2 >95%)	24 [18.5-39.6]	14 [9.5-54.2]	30 [17.4-36.4]	0.50
Data presented as Median [IQR]; IQR: interquartile range. Data analysed using Kruskal-Wallis test				

5.4.3.2. SpO_2 histogram

Figure 5.6 shows pooled histograms for SpO_2 during all the PDSA cycles. Comparing the first cycle, both the second and third cycles obtained improvement in increasing time within desired target range. It is interesting to note that third cycle showed a slightly shift to the right after changing lower alarm limit. The percentage of time SpO_2 below 80% was reduced during the second and third cycles.

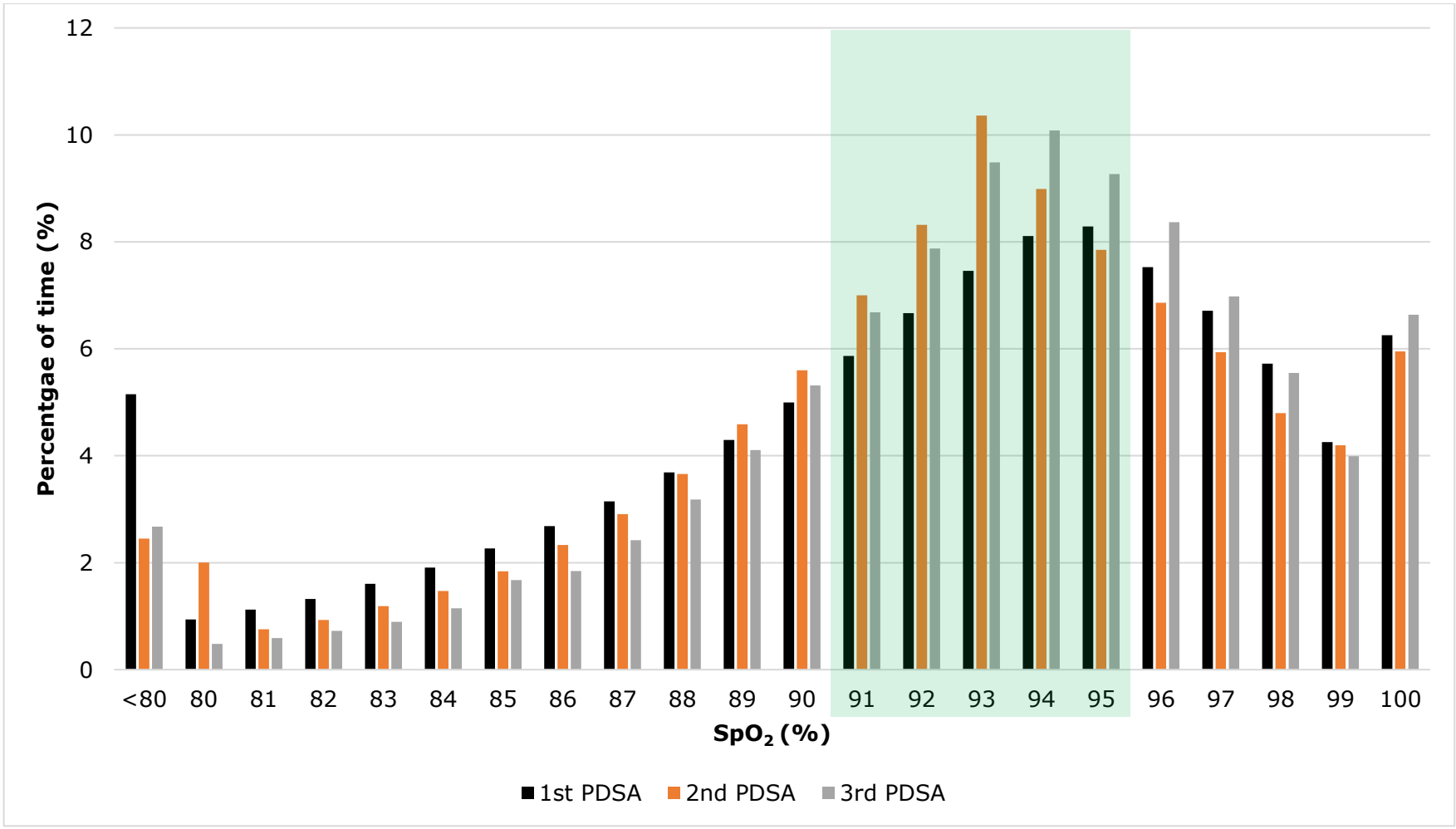


Figure 5.6 Composite SpO_2 histogram of all infants' data (n=37). The frequency of SpO_2 values indicate the percentage of total time spent at each SpO_2 with aggregate SpO_2 values <80%. The target SpO_2 range infants receiving oxygen therapy (91%-95%) is the shaded green rectangular.

5.5. Discussion

The NeoSat project set out with the aim of assessing and improving the percentage of time achieving targeted SpO_2 for preterm infants with gestational age <33 weeks receiving oxygen therapy. The project demonstrated a significant improvement in targeting oxygen saturations and increasing HCP compliance with setting SpO_2 alarm limits correctly.

An important finding of this QI project was the percentage time in the target range significantly increased from 34% to 40%, and this increase was achieved mainly as a consequence of reducing the time below target range with a shift of the SpO_2 histogram to the right. These findings differ from previously published quality improvement studies [112, 266, 267]. Ford *et al* reported that the percentage of time within their desired target range (90 to 94%) increased significantly from 20% to 35% compared to 34% to 40% in presented study, the time above targeting range (>94%) dropped from 78% to 40%, and the time below the wider targeting range, (i.e. 85 to 95%), which reported in this study as wider targeting range that different from the desired range increased, but not statistically significant, from 5% to 13% [266]. Moreover, Ford et al reported that time within the wider target range between 85 and 95% was significantly increased from 22% to 55% [266]. Lau *et al* did not report time within or below the target range, but did report time above this range (SpO_2>95%) which had reduced following their initiatives from a range of 40% to 60% in baseline to range of 20 to 50% after phase 1 and then subsequently to 0% to 15% after phase 2 [267]. However, Lau did not report any statistical test comparisons and therefore report percentage of time as ranges rather than exact average. Van Zanten *et al* reported that time within their target range (85% to 95) significantly improved from 48% to 61.9% and above targeting range significantly reduced from 44% to 30.8%, and time below targeting range remained similar [112]. A possible explanation for these differences could be methodological factors, as all the stated three studies implemented guidelines for

oxygen titration and educational sessions simultaneously. The implementation of guidelines with education could mask the effectiveness of education sessions as a stand-alone factor. In addition, no objective measurements were reported to identify the effectiveness of the educational sessions. Importantly, the SpO_2 recordings were using an average of different period that range from a one day to whole minute. The use of averaging could mask the accurate variation of what occur for infant in second bases and therefore could underestimate the number of time SpO_2 spent out the target range. Detail methodological approaches for these studies are summarised in Table 5.10.

Table 5.10 Quality improvement approaches used in previous SpO_2 targeting projects

	Ford [266]	**Lau [267]**	**Van Zanten [112]**
Study population	VLBW infants on oxygen therapy	<32 weeks gestational age and <1,500g on oxygen therapy	<30 weeks gestational age on respiratory support and oxygen therapy
Sample size	All infants admitted during project with no exact number mentioned	37 infants	136 infants
Study approach	PDSA cycle started with assessment of baseline measurements, followed by implementation of oxygen saturation targeting protocol and educational sessions. At the end data collection performed to assess changes	PDSA cycles started with baseline measurement followed by awareness and educational session and measurements in phase 1. FiO_2 titration guidelines then implemented in phase 2	Measurements before and after awareness and FiO_2 titration guidelines implementation were collected
Measurements	Daily mean SpO_2 range calculated for each infants	Record SpO_2 value at 15 minutes intervals manually	SpO_2 extracted every minute from patient data management system
SpO_2 target range	90% to 94%	85 % to 93%	85% to 95%
SpO_2 alarm limits	85% for lower limit 96% for upper limit	Unavailable	84% for lower limit 96% for upper limit

Which part of the presented QI project has contributed the greatest to target appropriate oxygen saturations is unclear and I will discuss the impact of each intervention.

5.5.1. Impact of educational package on SpO_2 targeting

The educational package was aimed at addressing the specific knowledge deficits identified in the pre-survey. The educational content includes feedback of baseline SpO_2 target results and information materials based on published evidence defining the importance of maximising optimal saturation management. The post-survey revealed that the educational strategy may not have been significantly effective and this raises questions as to why? It is rational to ask if the lack of improvement in knowledge was enough to influence HCPs practice, if the educational package was appropriate, and if the educational method could support a sustainable change in knowledge.

Previous studies that have used educational sessions as the only intervention to improve SpO_2 targeting observed that percentage of time spent within the target range improved by only by 1% [109]. It is surprising that Adrawarian *et al* found that the targeting time decreased, after educational sessions, by 4% and the time spent above target range increased by the same [102]. Deuber *et al* reported that an educational strategy aimed at reducing hyperoxaemia was not successful with the time above target range increasing post-education, the time spent within appropriate target range was not reported [273]. These previous reports were less successful compared to the present study. There are many factors that could have impacted the effect of educational sessions including a difference in educational content, methodology, and training duration. During the second PDSA cycle, where the educational session is the only intervention implemented, the HCPs' behaviour changed in a positive way. This was confirmed by an increase in compliance with setting the SpO_2 alarm limits correctly, and increasing the time in the target SpO_2

range. These might suggest the NeoSat educational sessions could be considered more effective compared to similar studies.

5.5.2. Impact of narrow alarm limits on SpO_2 targeting

One of the project objectives was to increase the compliance of correctly setting the SpO_2 alarm limits. Only once alarm limits are set correctly can you aim to improve target saturations. The improvement observed in the NeoSat project by narrowing alarm limits to allow better targeting of SpO_2 mirrored findings of previous studies that reported narrow alarm limits [98, 274].

The current finding in NeoSat project further supports the idea of using narrow alarm limit to increase the percentage of time within target range as observed in an earlier prospective multicentre cohort study [98]. It found that centres that use narrow alarm limits, defined as upper limit not exceeding 1% above the upper target range and lower limit not more than 2% below lower target range, have successfully maintained SpO_2 within intended range for time ranges between (42% to 64%) compared to other centres use wider alarm limits for time ranges between (16% to 58%).

A recent clinical study, compared the impact of smaller target range (i.e. 90 to 95%) versus wider range (i.e. 85 to 95%) on the compliance of SpO_2 targeting while maintaining the alarm limits directly below or above set target range [275]. The study found that SpO_2 distribution significantly shifted to the right with a median of 93% during wide range compared to 94% during small range. The time of SpO_2 between 90% and 95% were not significantly differed between two interventions, 49% during wider range and 47% during smaller range. It is interesting to note that during both interventions of this study, implementation of narrow SpO_2 alarm limit associated with keeping SpO_2 in the set range approximately half of the time and the SpO_2 time between 90% and 95% not significantly differed. It is hard to relate the unchanged in SpO_2 targeting time observed in this study to the narrow alarm limit used during both interventions.

However, a chance of this to be the cause could not be ignored specifically when compared to my finding and previous ones [98, 274].

Klevebro *et al* performed a cohort study to compare a higher saturation target of 90-95% with narrow alarm limits of 89-96% to lower saturation target of 88-92% with wider alarm limits of 85 to 95% among infants <32 weeks of gestational age. They reported that very narrow SpO_2 alarm limits could improve targeting but also could significantly increase time above 95% from 20% in lower target cohort to 28% in higher target cohort [274]. Interestingly, the NeoSat project did not significantly increase the time of hyperoxaemia. The percentage of time above the target range after narrowing the alarm limits resulted in non-significant increase compared to the baseline. Although it is not a significant, it may suggest that the educational sessions were effective in preventing the significant shift of SpO_2 histogram to the right after narrowing the SpO_2 alarm limits.

5.5.3. Compliance in setting SpO_2 alarm limits correctly

The intermittent audit of the SpO_2 alarm limits during all three PDSA cycles indicated that alarms were set correctly 56% at baseline and this significantly improved to 88% during third PDSA cycle. This improvement was mainly attained after educational sessions and reminder stickers containing the new SpO_2 alarm limits placed on every nurse clipboard.

The compliance of setting appropriate SpO_2 alarm limits improved as the QI project progressed, and this improvement could be as a result of one of two factors. The first factor is the increase in HCP awareness and attitudes to anticipated consequences of setting the SpO_2 alarm limits incorrectly. The second factor associated with the tendency of HCPs to set alarm limits correctly due to their awareness of being watched as the QI was on run, this was previously reported [276]. When infants participated in the BOOST II trial, more SpO_2 alarm limits were set correctly for infants participating in the study compared to those who weren't in the study [276]. The time of collection of the alarm limit data was not set and

staff nurses were not informed such data was being collected. Therefore, it is unlikely the alarm limits were modified in anticipation of audit rounds.

The NeoSat project found that the SpO_2 upper alarm limit was the most frequently incorrectly set, this finding was observed in earlier studies [108, 276]. Clucas *et al* [108] and Laptook *et al* [109] related this to infants with minimal oxygen requirement fluctuating from air to low-level FiO_2. However, it was not possible with the present study to replicate this analysis as alarm setting data was not collected in parallel with exact FiO_2 data. Moreover, a possible explanation based on my results is related to the HCPs knowledge level as they successfully identify ROP as the only condition associated with hyperoxaemia, during baseline measurement. In other hand, most HCPs related death, BPD, NEC, and IVH to hypoxaemia. Therefore, the observed underestimation of hyperoxaemia risk may lead to the majority of setting upper limit incorrect in NeoSat project.

5.5.4. Automated oxygen control system vs. manual SpO_2 targeting

In recent years, studies on the use of FiO_2 closed-loop systems (automated) for targeting SpO_2 in preterm infants' have increased [103, 104, 277, 278]. These trials reported that using automated systems significantly increase the time within the target range by approximately 10-30%, percentage of time ranged from 58% up to 80%. However, the percentage of time above target range varied among these studies. Two studies showed almost no change in time between automated and manual systems [103, 277]. Gadjos *et al* reported that percentage of time above desired target range was 4.05% during automated control and not significantly differed from manual control with 5.8% [103]. A recent study showed that despite the improvement in the percentage of time targeting SpO_2 and reducing the percentage of time out optimal range during automated monitoring, there were more hyperoxaemia episodes but shorter in duration in the automated compared with manual titration (37 episodes with average duration of 12 seconds during automated system vs. 18 episodes with average duration of 48 seconds

during manual system, P<0.0001) [104]. Zapata *et al* showed that an automated system could decrease the time spent above target range [278].

Although automated systems of oxygen delivery have been developed, they may not be the ideal way to keep SpO_2 in the target range. They have a number of drawbacks, including: 1) cost, with the majority being built into mechanical ventilators, 2) the response of increasing the FiO_2 is not always the most appropriate action to perform such as hypoxaemia caused by hypoventilation or airway blockage, 3) they reduce the attention of HCP and delay the recognition of respiratory or other clinical deterioration, and 4) they depend on the accuracy of the pulse oximeter, but the presence of motion artefact may influence the system's reliability. Further improvements to these automated algorithms in future could potentially fulfil the exact need for monitoring and supporting preterm infant's oxygenation.

5.6. Study strengths and limitations

5.6.1. Study strengths

A strength of the NeoSat project was the use of a PDSA approach that enabled formation of an intervention and subsequent measures to see if the problem had improved and identify further challenges that needed addressing. Another strength of the study was that SpO_2 values were extracted every second from clinical monitor without the use of any masking such as averaging time of different values, as has occurred in previous studies [112, 266, 267]. Moreover, NeoSat project collected and analysed approximately 7,500 hours of SpO_2 data, which enhance the precision of findings.

5.6.2. Study limitations

The findings in NeoSat project are subject to several limitations, first and importantly is the Hawthorne effect [279], which is described as HCPs behaviour alteration due to their awareness of being watched, and this could happen when the infants monitor is connected to the study system. The second limitation occurs

when conducting the pre/post-surveys, both surveys had a low response rate despite the efforts in minimising survey time to less than 5 minutes and sending several reminders. The third limitation is the inability to track HCPs who attended educational sessions, despite the effort made to repeat the educational session on a daily basis. It will be useful for future audits to form an attendance list and update it every session. The fourth limitation is the freezing of OxyGUI systems where some time points are missing. The fifth limitation is the inability to include preterm infants on room air in the analysis, as I noticed that during the first cycle when FiO_2 analyser disconnected a value of 0.21 (i.e. room air) recorded in the system despite that the infants on oxygen therapy, and the majority of infants during the second and third cycle were on oxygen therapy with few room air measurements. A final consideration is that as NeoSat project is QI study, no additional infant data were collected. Therefore, it is not possible to explore if the difference in patient characteristics contributed to the distribution of SpO_2 values.

5.7. Conclusion

The main findings of this project suggests that QI could be used to improve SpO_2 targeting. Combining HCP education with narrow SpO_2 alarm limits improved the compliance of keeping SpO_2 within target range in preterm infants <33 weeks gestational age. Educational sessions also improved the correct setting of SpO_2 alarm limits. This study has demonstrated the QI approach can improve the care of high-risk preterm infants, at least in the short-term.

5.8. Future work

Although the NeoSat project obtained noticeable improvement in SpO_2 targeting, several questions were raised while conducting this project. First, the sustainability of the improvement in oxygen targeting was not assessed, as time did not allow further evaluation. It is well known that the beneficial effect of educational sessions can decline with time [280]. Therefore, integration of SpO_2 targeting educational sessions into the NICU education programme, along with additional PDSA cycles

are required to allow assessment of the sustainability. Second, it would be worthwhile to conduct systematic review that critically appraise all quality projects used to improve SpO_2 targeting and identify the most successful approaches. Finally, if extended to include larger numbers then measuring the incidence rate changes of BPD or ROP during QI periods may help identify the impact of oxygen targeting on these important morbidities.

Chapter 6. Recording and utilisation of routine vital signs to predict respiratory deterioration in preterm infants: A feasibility observational study

6.1. Overview

Early warning systems, described earlier in the introduction chapter, are widely used to identify clinical deterioration. However, there are many factors that may impact the effectiveness of these systems, such as the inaccuracies of recorded data and scoring, reducing the opportunity to recognise early deterioration. Furthermore, HCPs compliance with the completion of these charts may also impact the effectiveness of these systems. Electronic recognition of clinical deterioration could facilitate the development of machine learning algorithms in neonatal care could help overcome the drawbacks of early warning chart systems. However, it is essential to identify the current shortcomings in clinical practice and explore patterns that could predict deterioration in the pre-machine learning phase before moving to the next stage (i.e. development of machine learning system).

Blood pressure (BP), HR, RR, and temperature are routinely collected vital signs on NICU. They are valuable measures used to determine a patient's condition during hospitalisation [281]. In addition, the pulse oximeter is used to determine the fifth vital sign, SpO_2 [282]. HCPs use vital signs as an important component of their clinical evaluation when trying to predict deterioration and a number of studies have identified the vital sign trend is beneficial as an early warning indicator of clinical status [283]. In the preterm population, additional events such as the pattern of desaturations and bradycardias could also help predict impending clinical deterioration.

Clark *et al* tested the hypothesis that a statistical model of bedside physiological monitoring data could detect respiratory decompensation for VLBW infants [122]. They collected vital sign parameters (SpO_2, RR, and HR) and waveforms (electrocardiogram, chest impedance pneumograph, and pulse oximetry

plethysmography), and completed a series of cross correlation and logistic regression measures to predict decompensation. The major clinical characteristic associated with respiratory deterioration was oxygen desaturation. However, any statistical model is designed mainly to find the relationship between variables and determining the significance between them, statistical modelling is explicit to math computations and programming and, therefore, the prediction attained [284, 285]. By contrast, machine learning focuses on the prediction by using a learning algorithm to find patterns in data.

The physiology of a patient undergoes changes prior to clinical deterioration [286]. In the NICU, bradycardia and desaturation events are common in preterm infants [287]. They activate NICU monitor alarms and attract clinical attention, the recording of the event frequency and severity depends on manual chart documentation [288]. Therefore, I propose the use of an electronic system, to continuously monitor dynamic changes in bradycardia and desaturation events, for assessing the reliability of manual chart documentation and identify any patterns prior to respiratory deterioration in preterm babies. Early identification of respiratory deterioration will allow HCPs to intervene in a more timely fashion and potentially reduce the incidence of complications associated with escalation of respiratory support i.e. BPD.

Use of automated systems for delivering oxygen to preterm infants has been shown to be superior to the manual titration method in targeting oxygen saturation and reducing time out of target range [289]. Automated systems record SpO_2 electronically and response to any change in SpO_2 by adjusting FiO_2 to keep saturation within the desired target range. The same algorithms that optimise target oxygen saturation could also be integrated into learning protocols to identify earlier deterioration.

6.2. Study aim and objectives

6.2.1. Aim

Identify 'physiological signatures' for individual preterm infants that could help predict early cardiorespiratory deterioration.

6.2.2. Objectives

1) Assess HCP chart documentation of bradycardia and desaturation events and compare this to an electronic record.

2) Explore early bradycardia and desaturation patterns of preterm infants that could predict respiratory deterioration.

6.3. Materials and methods

6.3.1. Theoretical model of feasibility study

Global health care systems should implement evidence based interventions that have been thoroughly evaluated. To accomplish this, a feasibility study of any new intervention is needed to determine whether a more detailed efficacy study is needed or if further modification is required prior to evaluation and implementation [290].

The proposed study evaluates the possibility of a computer to learn about individual preterm infant's cardiorespiratory events and identify when there is a difference from their normal pattern or behaviour. Therefore, the clinical care team could intervene earlier and improve short and long-term outcomes for the target population.

The recording of large new digital data and significant advances in computing power provide an opportunity to use a large amount of patient data and drive the future of clinical development through identifying meaningful clinical pattern using ML algorithm.

6.3.2. Study Design

This is a prospective observational study of preterm infants at risk of respiratory deterioration. The study was conducted in two tertiary NICUs at Nottingham University Hospitals from August 2018 to August 2019. Clinical and vital signs data were analysed offline.

6.3.3. Study population

The study population included preterm infants requiring admission to the NICU and undergoing continuous vital sign monitoring. The inclusion criteria consisted of preterm infants born with gestational age less than 37 weeks, undergoing continuous vital sign monitoring, on non-invasive or invasive respiratory support and with written informed parental consent. Infants undergoing palliative care were excluded.

A convenience sample was used in the study when the researcher was available to recruit and set up the monitoring system. The study was explained to the infant's parent and a study information sheet was given to them (Appendix 21) prior to signing the consent form (Appendix 22).

6.3.4. Study regimen and data collection

At study entry, data to confirm eligibility as detailed above and informed consent were obtained. For each participant recruited, routine vital sign data and clinical observations, patient demographics and maternal clinical details were collected from the medical records. Routine observations of SpO_2, HR, FiO_2, mode of respiratory support, number of bradycardic events and number of desaturation events were recorded hourly in the nursing care chart as part of normal care. The nurse recorded the lowest bradycardia or desaturation event on the observation chart for HR or SpO_2. These measurements were collected and recorded in the study case report form.

The study used the OxyGUI system (described in detail in chapter 5) to continuously collect SpO_2, HR, and FiO_2 data from the bedside monitors. The patient vital sign monitor (GE Carescape) was attached to the infant as per the normal care pathway dictated by the clinical team caring for them. The oxygen analyser was incorporated into the participants breathing circuit. All information was recorded anonymously and labelled with a unique study number. The OxyGUI software collected HR data from electrocardiogram, SpO_2 from pulse oximetry, and FiO_2 from oxygen analyser, every second. Bradycardia and desaturation events were collected for each infant during the study to investigate the accuracy of manual charts compared to electronic episodes.

Each participant was involved in the study for the duration of their requirement for continuous monitoring and need for MV or non-invasive ventilation. Infants successfully maintained off positive pressure respiratory support for 24 hours were deemed to have completed the study.

6.3.5. Definitions of bradycardia, desaturation and escalation events

For the purpose of this study, the following definitions were used: bradycardia was defined as drop in the HR to less than 100 beats per minute for ≥4 seconds; desaturation was defined as drop in SpO_2 to less than 80% for ≥10 seconds [288]. There was no policy or guideline in place to define bradycardia and desaturation events for nurses to document these on patient charts.

Escalation events were defined as non-elective initiation of MV as deemed necessary by the attending clinical team. The causes for escalation include worsening respiratory distress and increasing oxygen requirement. Elective events included planned surgery requiring intubation or transfer for imaging.

6.3.6. Data preparation

The frequency of bradycardia and desaturation events documented in nursing charts was recorded in the study case report forms. Events were transferred into a Microsoft excel file format to compare it with the electronic recorded events. The electronic recorded data for each infant were used in the analysis after excluding duplicates and data with poor signal quality defined by the signal quality value of 0 (i.e. artefact HR which defined as quality value= 1 and HR =1, incorrect SpO_2 value when probes disconnected which was reported by the monitor as SpO_2 value of 327).

R programming scripts were written by Dr Andrew Prayle to identify the defined bradycardia and desaturation events from the electronically recorded data. The validity of the R programing script was checked, by a manual quantification of the number of bradycardia and desaturation events for all escalation days and compared with R written commands output. The manually counted events for each escalation day were identical to that obtained via R commands (Appendix 23).

The pattern of bradycardia and desaturation events were studied during the 24-hour period before escalation as detailed in the nursing documentation. The hour that the escalation occurred was not included in the analysis due to uncertainty of

the exact escalation time. The 24 hour period was used as priori determined by HeRO monitoring system to predict clinical deterioration caused by an infection within 24 hours [291].

6.3.7. Ethical consideration

This study was approved by East Midlands, Nottingham 1 research ethics committee, REC reference number: 18/EM/0033.

6.3.8. Statistical analysis

Events were made into hourly blocks to allow comparison between electronic and manual chart documentation. The Shapiro-Wilk normality test revealed data were not normally distributed; therefore the median number of manually recorded events during the study period were paired with the median number of electronic recorded data. Statistical comparison used paired Wilcoxon signed-rank test.

The 24-hour period before respiratory escalation was divided into two 12-hour blocks and the number of electronic events (i.e. bradycardia and desaturations) were paired for each escalation event. Statistical comparison between these two periods was conducted using either paired t-test for parametric data or paired Wilcoxon signed-rank test for non-parametric data following assessment of normality by the Shapiro-Wilk normality test.

Findings are reported as median [IQR], unless otherwise noted. All statistical analysis and graphs were prepared using GraphPad Prism V8 (GraphPad). A P value of <0.05 was considered statistically significant.

6.4. Results

6.4.1. Time series data

14 preterm infants were recruited providing 437 days of data. The median length of study per patient was 21 days [IQR 3-76]. The detail of each participant's duration of study, postnatal age at start of study, and number of escalation events are shown in Table 6.1.

Seven infants required respiratory escalation with a total of 13 escalation events occurring during the study period, three escalation events were excluded from the analysis as two were deemed elective (i.e. intubation before surgery) and one escalation event was missing a significant quantity of electronic recorded data due to a technical error. All escalation events were due to increased cardiorespiratory instability, as described in the medical notes. Detailed clinical information for each escalation is attached in Appendix 24.

Table 6.1 Detail of participants describing duration of study, age at study entry, and escalation events

Participant number	Duration of study (days)	Postnatal age at recruitment (days)	Number of escalation events
1	5	5	0
2	15	9	1
3	72	2	2
4	5	1	0
5	10	1	0
6	38	3	1
7	37	1	1
8	14	2	0
9	3	2	0
10	76	5	3
11	26	3	2
12	59	3	3
13	68	2	0
14	9	1	0

6.4.2. Demographic characteristics

14 preterm male infants were recruited into the study during the study period (Table 6.2).

Table 6.2 Preterm infants' demographic characteristics

Infants' characteristics (n=14)	
Gestational age in weeks	27 (26-30.25)
Birthweight in grams	1005g (790-1591)
Age at time of study in days	2 (1-4)
Weight at time of study in grams	1043g (796-1601)
Data presented as Median (Interquartile range).	

6.4.3. Documentation compliance of desaturation and bradycardia events

The 437 study days provided electronic data for 36,296 desaturation and 6,647 bradycardic events, these were found with good signal quality after removing motion artefact observations. In parallel, the documented charts provided only 1,976 desaturation and 1,693 bradycardic events.

The electronic recorder identified a significantly higher number of desaturation events compared to manual documentation per day per infant (33 [12-78] vs 1 [0-4], $p<0.001$) (Figure 6.1A). The electronic system recorded a significantly higher number of bradycardic events compared to manual documentation per day per infant (8 [4-11] vs 1 [0-3], $p<0.001$) (Figure 6.1B).

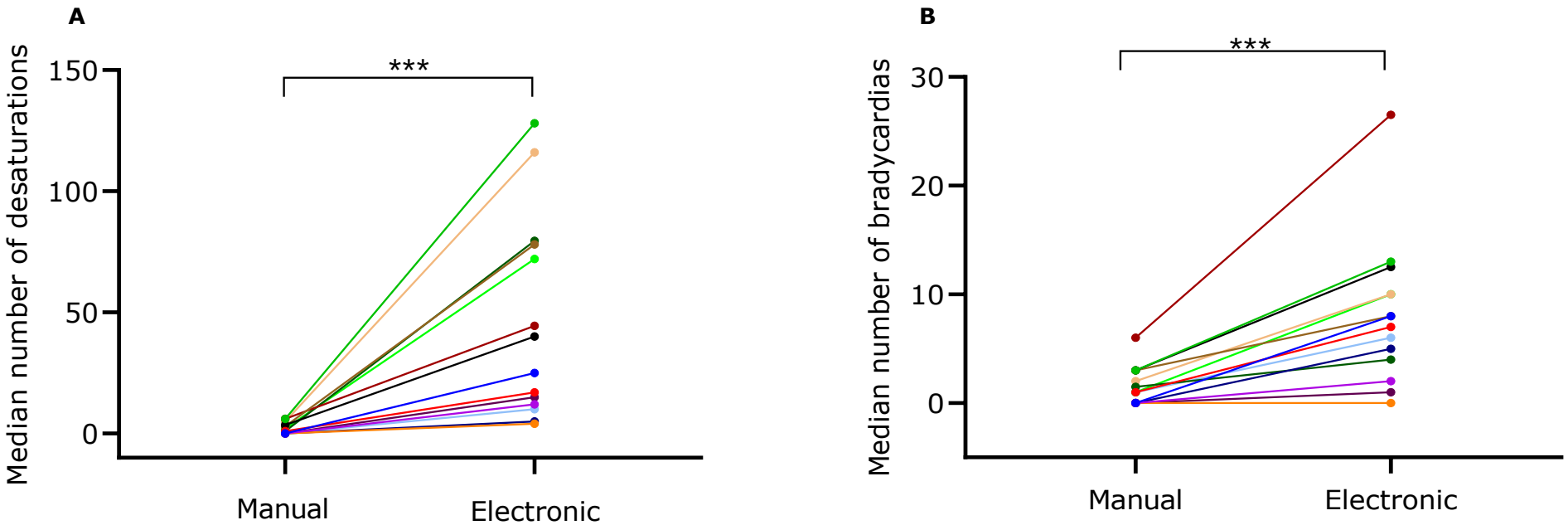


Figure 6.1 Comparison of the median number of daily desaturations (A) and bradycardias (B) for paired documented chart and electronic systems. Individual paired values are connected for each infant (n=14). ***P<0.001.

6.4.4. Pattern of desaturation events prior to respiratory escalation

During the study, there were 10 episodes of respiratory escalation captured. In the 24 hour period before escalation, the manual documentation revealed a similar finding with a significantly higher number of desaturation events during the second 12 hours period compared to the first period (10 ± 5 vs 5 ± 4, $p<0.01$ respectively) (Figure 6.2A). Likewise, the electronic data had a mean number of desaturation events that was significantly higher in the second 12 hour period compared to the first period (90 ± 54 vs 64 ± 54, $p<0.01$ respectively) (Figure 6.2B).

The hourly rate of desaturation events using manual and electronic records is presented in Table 6.3. It is interesting to note that the hourly rate of manual documented desaturation events was significantly increased during the second 12 hour block compared to the first 12 hour block (0.8 ± 0.4 vs 0.4 ± 0.3, $p<0.01$ respectively). The same significant difference was observed in the second 12 hour period with higher rate of electronically record desaturation events compared to the first 12 hours (7.5 ± 4.5 vs 5 ± 4.4, $p<0.01$ respectively).

Table 6.3. Hourly rate of desaturation events illustrating manual and electronic records per 12-hour block before escalation event.

Esc. event	1st 12 hours (manual)		1st 12 hours (electronic)		2nd 12 hours (manual)		2nd 12 hours (electronic)	
	Total	Rate	Total	Rate	Total	Rate	Total	Rate
1	0	0	5	0.4	12	1.0	57	4.8
2	9	0.8	136	11.0	21	1.8	154	13.0
3	2	0.2	8	0.7	8	0.7	24	2.0
4	3	0.3	9	0.8	4	0.3	16	1.0
5	11	0.9	122	10.0	13	1.1	131	11.0
6	4	0.3	100	8.0	16	1.3	162	13.5
7	4	0.3	40	3.0	8	0.7	76	6.3
8	2	0.2	76	6.0	9	0.8	96	8.0
9	1	0.1	17	1.4	5	0.4	45	3.8
10	9	0.8	127	10.6	5	0.4	135	11.3

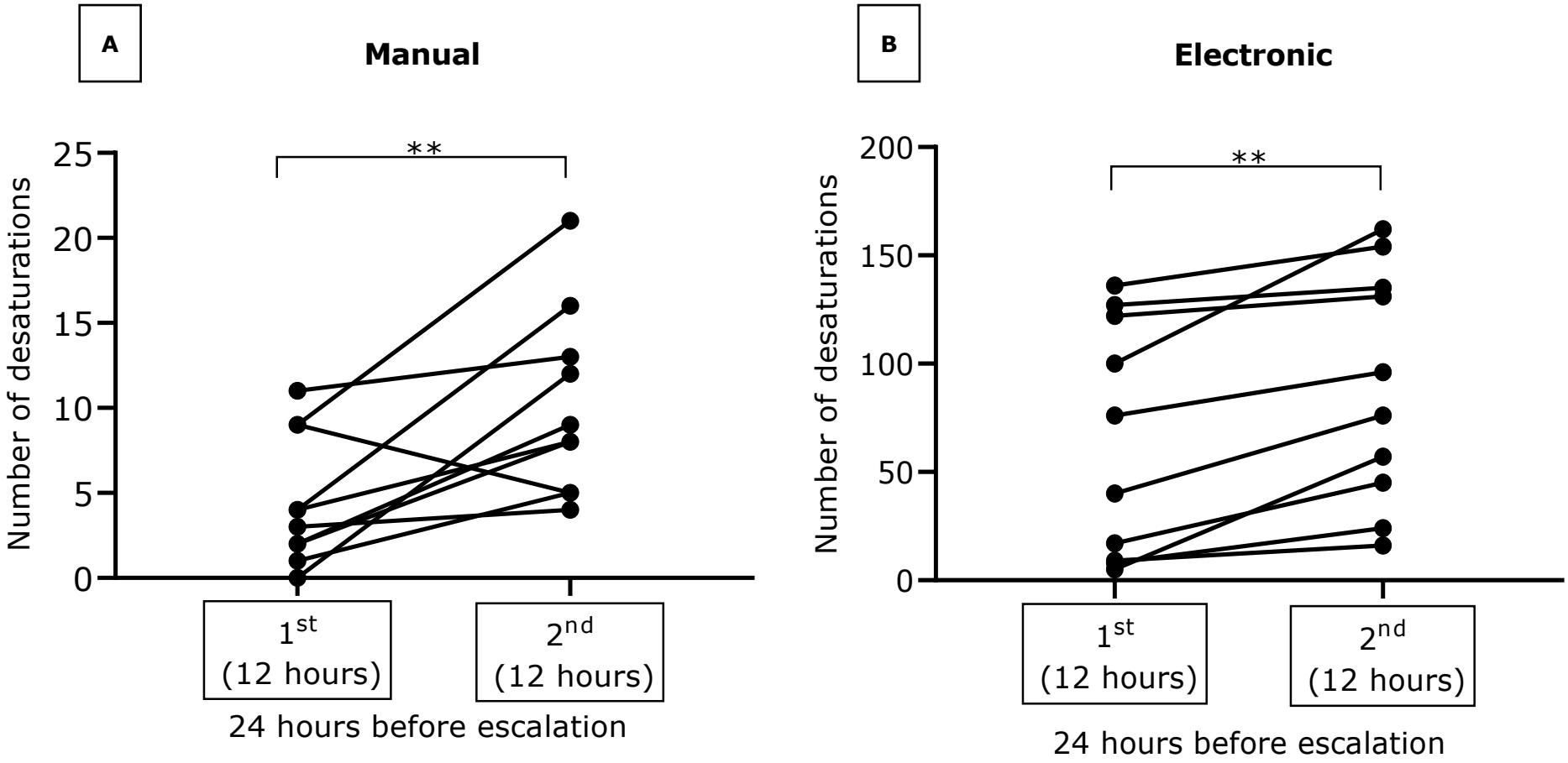


Figure 6.2 Comparison of number of desaturation events of individual patient episodes requiring respiratory escalation. (A) Manual observation chart documented desaturation events and (B) Electronic recorded. Individual paired values that connected by line indicate the same escalation event. **P<0.01.

6.4.5. Pattern of bradycardic events prior to respiratory escalation

The number of bradycardic events were significantly higher in the second 12 hour period compared to the first one for the manual chart method (12.5 [4-16] vs 4 [2-6], p<0.01 respectively) (Figure 6.3A) and for the electronic method (42 [16-59] vs 17 [9-23], p<0.01 respectively) (Figure 6.3B).

The hourly rate of bradycardic events using the manual chart and electronic recorder per 12-hour block prior to escalation is presented in Table 6.4. The hourly rate of bradycardic events manually documented is significantly increased during the second 12 hour period compared to first 12 hour period (1 [0.3-1.3] vs 0.3 [0.2-0.5], p<0.01 respectively). Moreover, the hourly rate of bradycardic events electronically recorded showed a significant increase during second 12 hour block compared to the first one (3.5 [1-5] vs 1.5 [1-2] , P<0.01 respectively).

Table 6.4. Hourly rate of bradycardia events illustrating manual and electronic records per 12-hour block before escalation event

Esc. event	1st 12 hours (manual)		1st 12 hours (electronic)		2nd 12 hours (manual)		2nd 12 hours (electronic)	
	Total	Rate	Total	rate	Total	Rate	Total	Rate
1	5	0.4	18	1.5	16	1.3	53	4.4
2	9	0.8	20	1.7	16	1.3	47	3.9
3	5	0.4	19	1.6	10	0.8	39	3.3
4	2	0.2	3	0.3	4	0.3	21	1.8
5	12	1.0	60	5.0	16	1.3	88	7.3
6	4	0.3	33	2.8	15	1.3	77	6.4
7	4	0.3	11	0.9	4	0.3	12	1.0
8	1	0.1	0	0	8	0.7	17	1.4
9	3	0.3	17	1.4	16	1.3	45	3.8
10	2	0.2	13	1.1	4	0.3	5	0.4

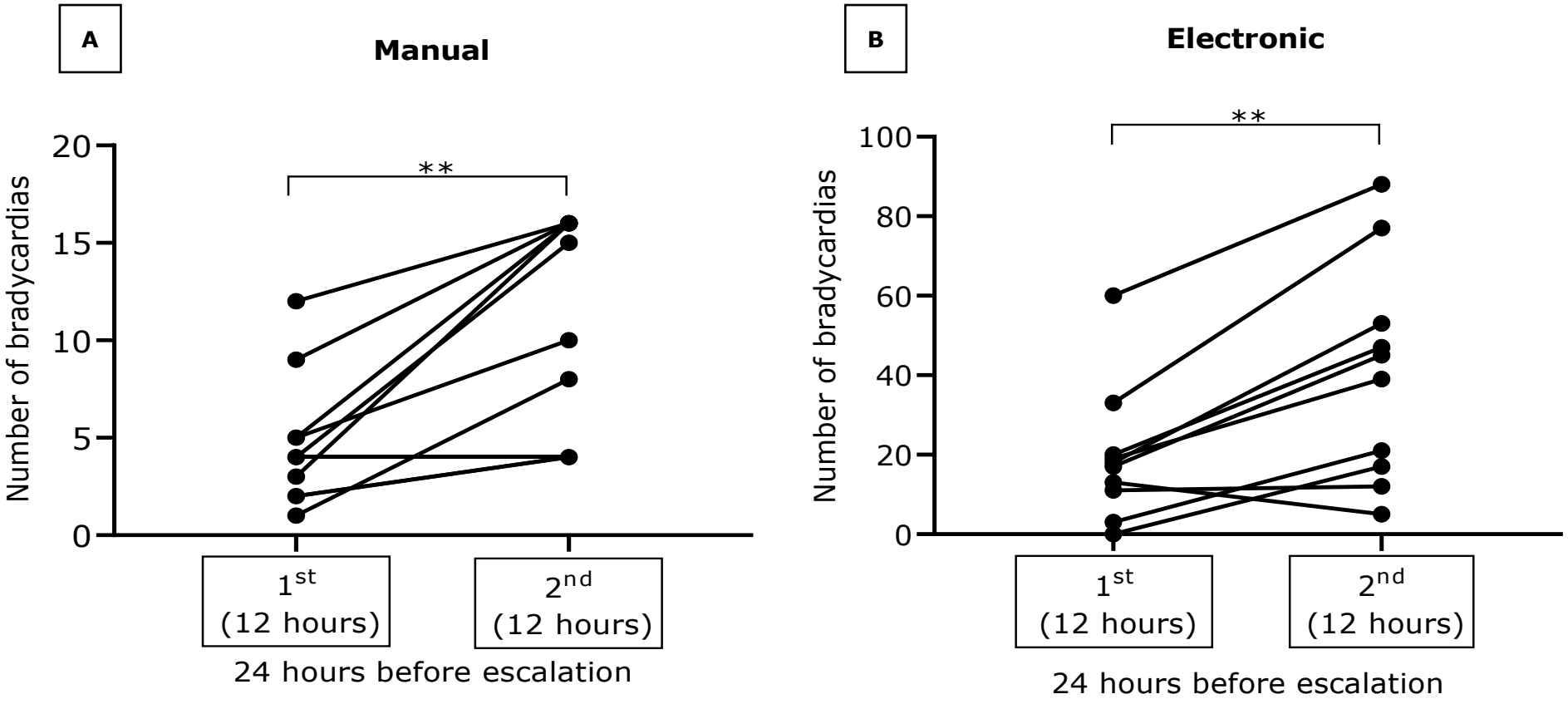


Figure 6.3 Comparison of number of bradycardia events of individual patient episodes requiring respiratory escalation. (A) Manual observation chart documented bradycardia events and (B) electronic recorded. Individual paired values connected by a line indicate the same escalation event. **P<0.01.

6.4.6. Example of SpO_2 data

Figure **6.4** is an example of 24-hour period prior to an escalation event. The patient was a preterm infant born at gestational age 26 weeks and recruited into the study on the second day of life. The electronic SpO_2 data shown in (Figure 6.4A) demonstrates an increase in the frequency of desaturation events during the second 12 hours period before respiratory escalation compared to the first 12 hours period. However, the manual trace of the SpO_2 records hourly average value of SpO_2, (Figure 6.4B).

Figure 6.5 presented hourly blocks of the number of desaturation events recorded electronically and manually during 24 hours before escalation for the same infant. The figures demonstrated variability in desaturation events recorded manually compared to electronically ones. It is worth it to note the increased of manual documentation as the infants deteriorated before escalation occurred.

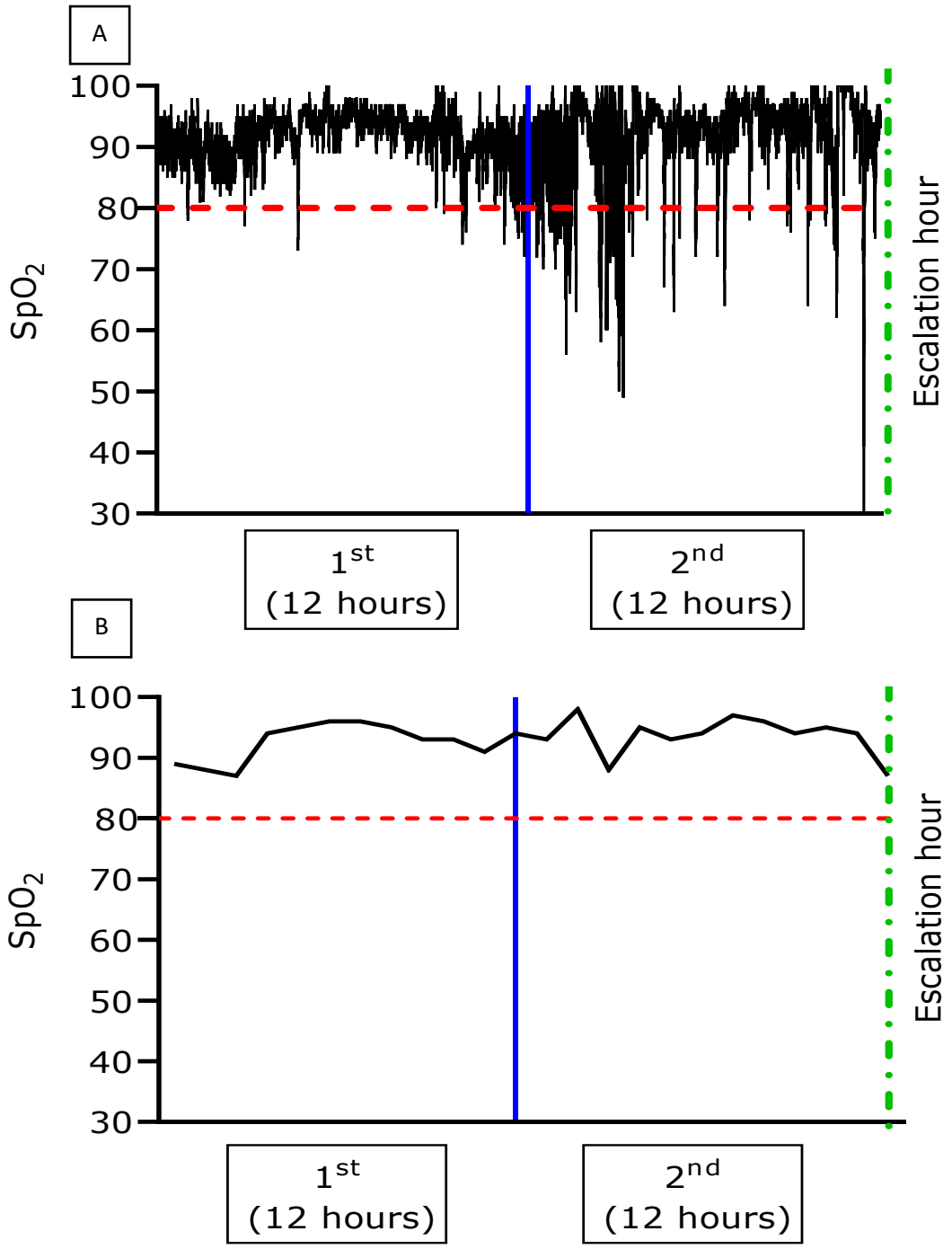


Figure 6.4 Example of complete 24 hours of oxygen saturation (SpO_2) recording before escalation for a 26-week preterm infant. Figure A represents electronic recorded SpO_2 and figure B represents manual recorded SpO_2. The red horizontal dot line indicates SpO_2 of 80% considered as the cut point for a desaturation event. The blue vertical line divided the trace into two identical 12 hours period. The green vertical dot line indicates the escalation hour.

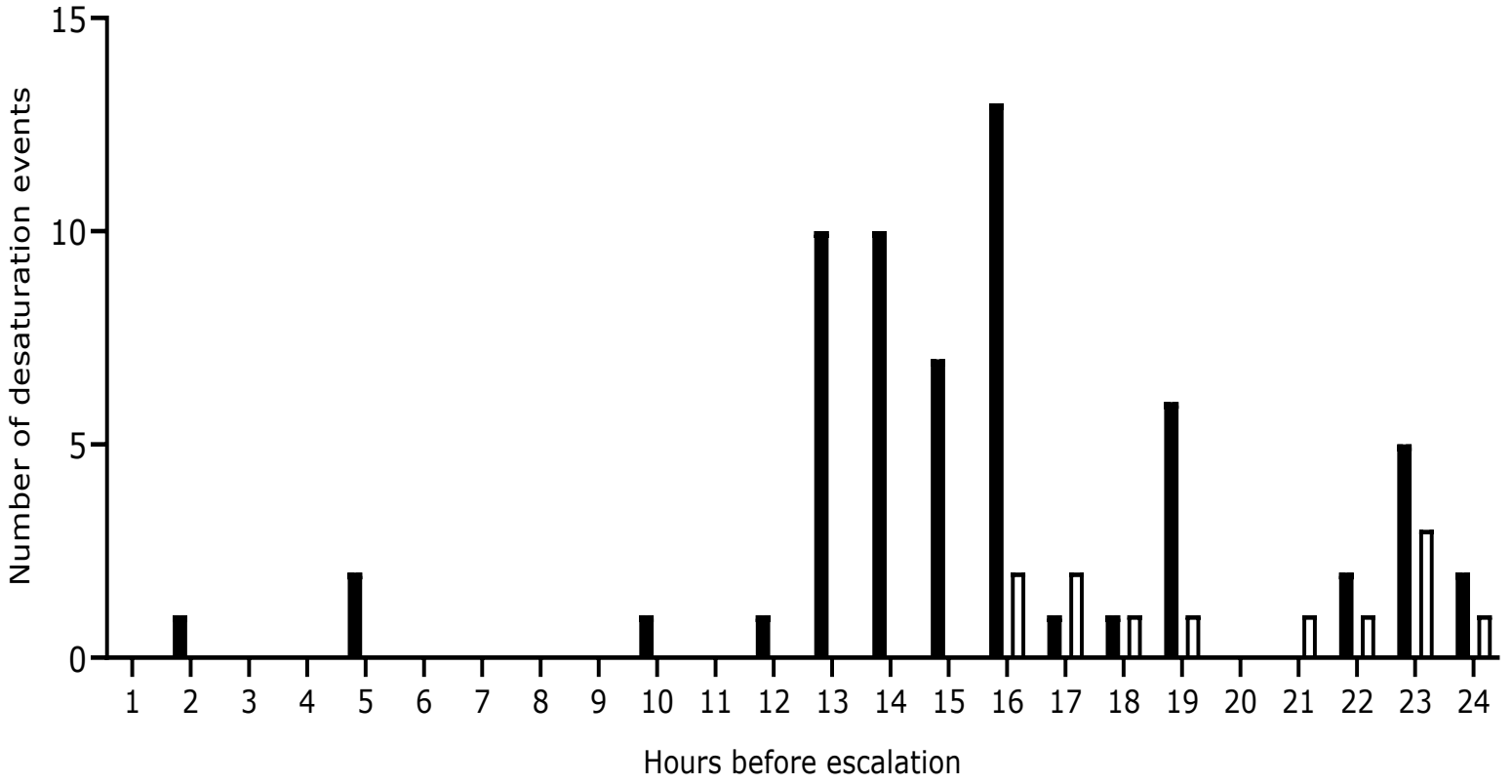


Figure 6.5 Complete 24 hours of desaturation events electronically recorded (black bars) and manually documented (white bars) before escalation for 26 weeks preterm infants.

6.4.7. Example of HR data

Figure **6.6** shows the HR trace before the escalation event for the same infant in section 6.4.6. The electronic record (Figure 6.6A) presented a higher number of bradycardias during the second 12-hour period compared to the first period. The manually documented trace of the HR reported hourly average values as shown in Figure 6.6B.

The hourly blocks comparing electronic and manual record of bradycardia events during 24 hours before escalation for the same infant showed an increase in manually recorded bradycardias as the infant deteriorated before escalation time (Figure 6.7).

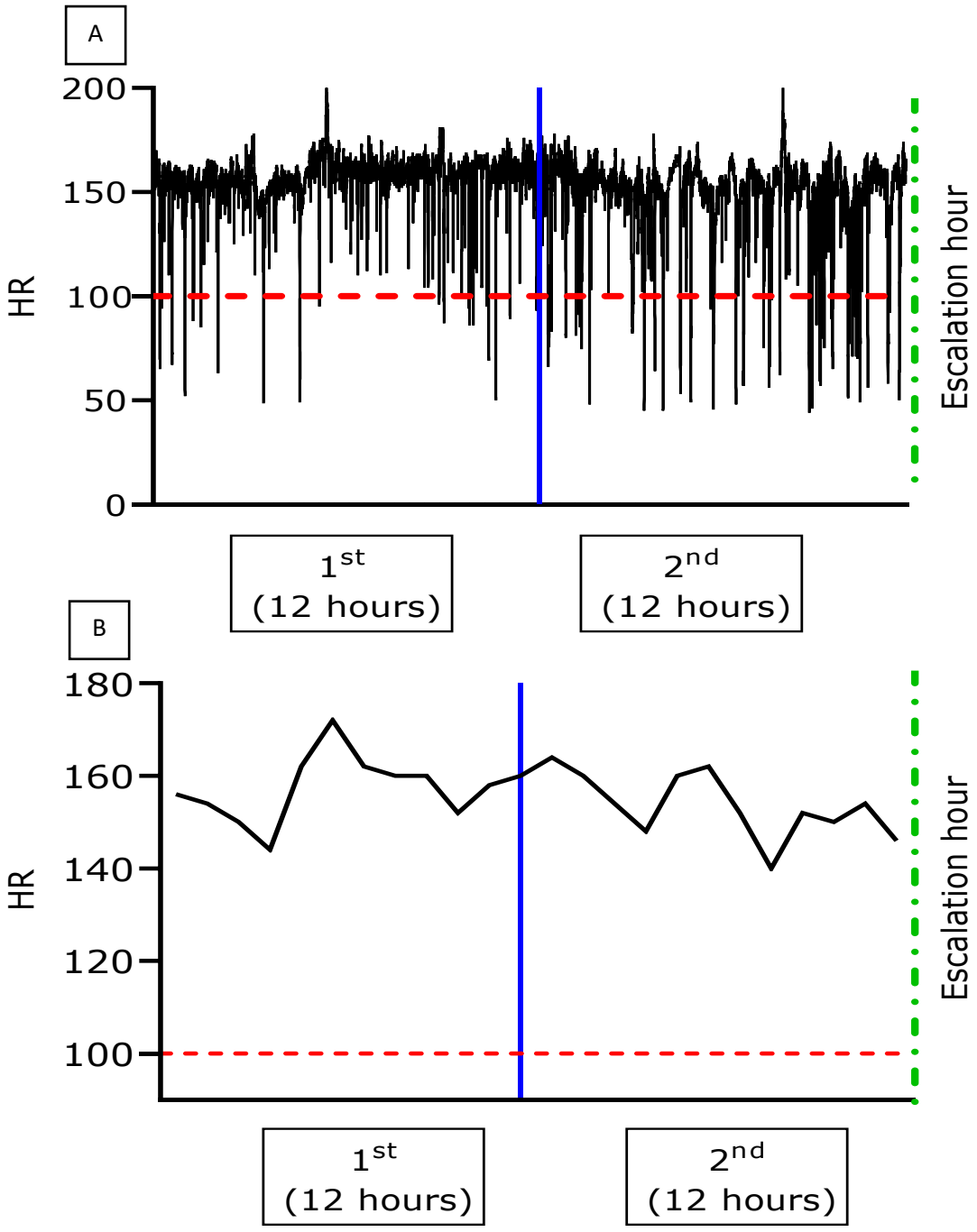


Figure 6.6 Example of complete 24 hours of heart rate (HR) recording before escalation for a 26 weeks preterm infant. Figure A represents the electronic recording of HR and figure B represents the manual documented chart of HR. The red horizontal dot line indicates HR of 100bpm considered as the cut point for bradycardic event. The blue vertical line divided the trace into two identical 12-hour periods. The green vertical dot line indicates the escalation hour.

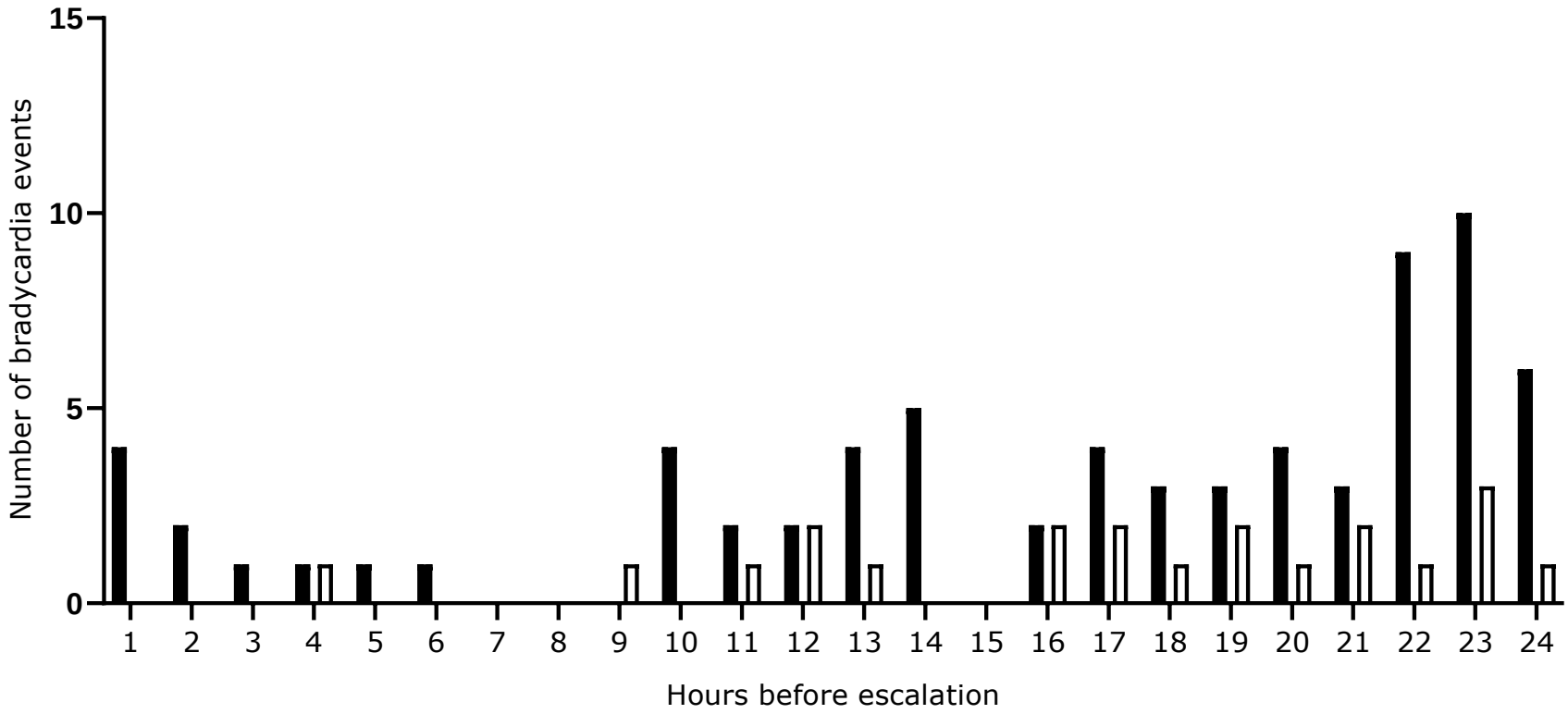


Figure 6.7 Complete 24 hours of bradycardic events electronically recorded (black bars) and manually documented (white bars) before escalation for a 26-week preterm infant.

6.5. Discussion

6.5.1. Comparison of manual documentation and electronic recording

Preterm infants undergo continuous monitoring of SpO_2 and HR during their stay in the NICU. The clinical team primarily depends on manual chart documentation of cardiorespiratory events for the evaluation and management of infants. My findings suggest that there is an inconsistency between the manual documentation and electronic records of bradycardia and desaturation events. Manual documentation underrepresents the frequency of both events with only 5% of desaturations and 25% of bradycardic events reported.

The present study findings are in line with earlier studies that conclude the superiority of electronic recording over manual documentation. Brockmann *et al* performed a comparison study to assess the accuracy of identifying bradycardia and desaturation events between manual documentation and an objective measurement tool using polysomnography in 21 preterm infants and showed that only 23% of desaturations and 60% of bradycardic events were documented [292]. Vergales *et al* performed a study comparing desaturation and bradycardia events documented manually with a computer algorithm from bedside monitors in 276 infants. They showed that 70% of electronically recorded events were not documented in nursing charts [293].

The discrepancy between manually and electronically recorded events may possibly be explained by the high clinical workload for nursing staff in the NICU. Heavy workload can result in inadequate time to document events [294, 295]. In addition, alarm fatigue could account for the low compliance in charting clinical events. It has been linked with unfavourable behaviours where clinicians decrease alarm frequency by setting inappropriate limits or silencing alarms which could hinder the documentation [296]. Moreover, HCPs may observe clinical events and consider it to be insignificant at that time or interpret it as not clinically relevant such as self-resolved events. Importantly, the discrepancy might be related to the absence of

clear guidance on when to record the events and most of the event documentation based on personal view. Therefore, a senior HCP could be more tolerant with significant events and not record them. Despite the effort made in the present study to exclude all the artefact observations it is possible some remained and contributed to the observed discrepancy.

The present study illustrates that the traditional way of event documentation underestimates the actual number, i.e. 95% of desaturations and 75% of bradycardic events, and therefore this may affect clinical decision making if using these in isolation. The use of electronic monitoring systems could improve the compliance of recording events and reflect the real cardiorespiratory stability of the infant. As HCPs often utilise desaturation and bradycardic events when assessing infants, better recording of these could improve the timely recognition of instability and enhance clinical management of preterm infants.

It is encouraging to link the current finding of the superiority of electronic monitoring system with that discussed in chapter 5 regarding SpO_2 targeting using automated versus manual systems. The automated system could significantly improve targeting oxygen saturation and show superiority to other manual systems [289]. One of important causes of the reduced documentation with the manual system is potentially the increased workload of HCPs where the team do not have time to document and struggle to maintain optimal saturation targeting [99]. It is therefore expected that use of automated systems in monitoring respiratory for preterm infant might be associated with more benefit in term of outcomes. However, the use of these systems should not be implemented in a rush without proper evaluation.

6.5.2. Cardiorespiratory deterioration prior to respiratory escalation

The second objective of this study sought to determine the pattern of bradycardia and desaturation events during the preceding 24 hours before urgent respiratory escalation occurred. This study used SpO_2 and HR monitoring to quantify the

frequency of bradycardia and desaturation in preterm infants before urgent intubation. The frequency of both events were significantly higher during the 12 hours before escalation compared to the previous 12-hour period. Although not an unexpected finding, the value of these findings is in the possibility of understanding respiratory deterioration using the pattern of bradycardia and desaturation events in the 24 hours before respiratory escalation for preterm infants.

The present findings of increased manual documentation of events prior to escalation, in keeping with a previous study aimed to assess the use of an algorithm that uses manually documented vital signs, bradycardia and desaturation events to predict earlier clinical deterioration caused by infection or NEC in the first month of life [297]. Their algorithm was able to detect clinical deterioration related to infection earlier than clinical suspicion of infection. Therefore, it could be possible that manual documentation could be used to predict deterioration. However, it is still not necessarily that manual documentation provided the real number of events and clearly illustrate the pattern of deterioration as electronic monitoring system.

Clark *et al* aimed to develop a statistical model to predict the need for urgent intubation in preterm very low birth weight (VLBW) infants. They found that desaturations, especially with bradycardia, were an independent factor predictive of respiratory deterioration [122]. However, this study was limited by using an averaging time of 10 seconds for recorded saturations and HR, in addition to the HeRO monitoring system that could support clinical decision making specifically for sepsis related intubation.

It is interesting to note that the frequency of bradycardia increased during the second 12-hour period and this may linked to increased hypoxaemia. The bradycardia could occur as a result of hypoxaemia triggering the peripheral chemoreceptor reflex [298]. Hypoxaemia stimulates the carotid chemoreceptor body, which can cause a vagal stimulation and reduction in sympathetic stimulation of the heart resulting in a bradycardia [299, 300]. Bradycardia and desaturation events in preterm infants are used clinically to identify changing infant condition,

this may well then support the need for the initiation of MV to stabilise the infant and oxygenation [301].

In the current study, comparing hourly rate of recorded events either manually or electronically during second 12 hour period compared with first 12 hour period showed that events were significantly increased during second period. This finding may be due to the increasing concern of HCPs regarding the status of the infant during the second 12 hours window where number of events significantly increases and alarms frequently trigger.

My study findings support the potential role of developing monitoring systems that could predict preterm infant respiratory deterioration based on bradycardia and desaturation patterns.

6.5.3. Development of a respiratory deterioration prediction system

The manual documentation underestimates the frequency of cardiorespiratory events among preterm infants. Moreover, the use of manual adjustment of FiO_2 reported previously in chapter 5 was not quite successful in significantly reducing time out the target range. The use of electronic monitoring system could offer advantages for HCPs to act as clinical decision support system and aid human interpretation of clinical deterioration. Integration of machine learning algorithms that learn about individual preterm infants from their vital sign patterns could be possible.

Bradycardia and desaturation events usually increase before clinical deterioration. Use of manual documentation may either fail to record or significantly underestimate the number of events because of alarm fatigue or differences in experience among HCPs. Therefore, manual documentation could hinder the HCP from picking out patterns well, especially if the rate, for example, events/hour is low and so recognising a pattern is difficult. The electronic recording does not fatigue or hesitate to record an event once it happens. Electronic recording captures many more events that could allow pattern to emerge. The pattern of events can

be hugely different between well and unwell infants, an artificial intelligence system could learn about the normal and changing patterns to trigger a warning to the clinical team. Such a system must be able to identify errors in the data such as events may be due to artefact.

The development of prediction monitoring systems need to move through many developmental stages. Initially there is a need to identify a potentially harmful outcome which early intervention could minimise and if there is a physiological signature that occurs before. My study focused on acute respiratory deterioration as the outcome and identified an increased pattern of these events as the physiological signature prior to this. In the future, gathering data and identification of other clinical features associated with respiratory deterioration, such as oxygen requirement, should be addressed. Finally, there is the potential to use the significant amount of labelled data to train a computer and then develop machine-learning algorithm to predict respiratory deterioration. An example of a previously developed approach is the HeRO monitoring system that described earlier in the introduction chapter and uses a different approach for earlier prediction of sepsis in neonatal settings [302]. Like this system, additional variables could be included in future models to optimise its function.

6.6. Study strengths and limitations

6.6.1. Study strengths

The strengths of this study are that the data collected by the OxyGUI system are routinely available in NICUs and so do not need additional sensors or equipment as the software can be incorporated into current devices. In addition, the OxyGUI system has the ability to differentiate between artefact and true cardiorespiratory events using the signal quality variable, and the efficiency of the system recording a significant amount of second by second data with minimal loss or interruption. No adverse events were reported when using the systems.

Additionally, extracted SpO_2 and HR data were not averaged over time, reflecting actual events occurring each second. Finally, the findings support the possibility of developing predictive monitoring that can detect impending respiratory deterioration to supplement HCPs decision making in high-risk infants.

6.6.2. Study limitations

There are several limitations to this study. The bradycardia and desaturation event definitions used from electronic system in the study may not match the nursing record used to document these events, in part because of the lack of a local guideline. The OxyGUI software used to collect patient monitor data did have some limitations. Technical issues occurred with one system resulting in that system being taken out of service for three months reducing data collection opportunities. The desaturation accompanying bradycardia was not reported in the present study, future work could develop the R command script to include this output. In addition, the FiO_2 analyser was disconnected from the breathing circuit numerous times, especially before escalation, and this limited the use of oxygen requirement in the analysis phase.

6.7. Conclusion

The continuous electronic record of HR and SpO_2 is superior to manual documentation for recording the frequency of bradycardia and desaturation events in preterm infants and could be a more reliable method used to detect trend changes when predicting infant cardiovascular instability.

Intermittent manual documentation underestimates the number of desaturation and bradycardic events. Therefore, the prospect of a monitor that detects desaturation and bradycardic patterns, learning about an individual infant may be beneficial in guiding bedside management.

In summary, the findings of this study support exploration of the potential to develop learning algorithms, such as those possible with machine learning, as the pattern of events may not be recognised from conventional approaches. The

development of such a system should not replace the clinical judgment of the healthcare team but help support their decision making to intervene at the appropriate time.

6.8. Future work

The recruitment for this study is ongoing with the aim of collecting additional machine learning training data for the next step of exploring the development of a prediction algorithm. A minor change in the inclusion criteria to focus on extremely and very preterm infants is recommended, as the present study found that escalation events occurred frequently for these patients. Additionally, there is the possibility of integrating the oxygen requirement pattern before escalation into the algorithm.

The present study limited the recruitment to preterm infants on either non-invasive ventilation or MV. However, expanding the population to include other infants, for example those not needing respiratory support or on oxygen cannula, need to be considered to understand the pattern of bradycardia and desaturation events for such patients.

The implementation of a large new digital data source, and the significant advance of computing power, provide a great opportunity for using large amounts of patient data. Clinical development of techniques identifying meaningful clinical patterns using machine-learning algorithms could represent a major step in patient management. The large dataset obtained from this study could be used as first phase training data to explore this further.

Chapter 7. Thesis Conclusions and future implications

7.1. Chapter overview

The studies presented in this thesis was undertaken to understand the challenges facing the respiratory management for extremely and very preterm infants. To achieve this it was important to understand current respiratory management and the incidence of BPD in England and Wales. The adverse impact of inter-hospital transfer in early life on severe morbidity such as IVH has been well studied but the effect on BPD is less clear. I undertook analyses to explore the potential association of BPD related to early life preterm infant transfer and supported these findings using a novel rodent model. Finally, my thesis discusses the challenges of optimising respiratory management for preterm infants in relation to minimising the risk of BPD and how this can be done at local level.

The current chapter summarises the main findings, discusses the general limitations, and overviews the clinical and research implications. A summary of thesis findings and contributions is shown in Figure 7.1.

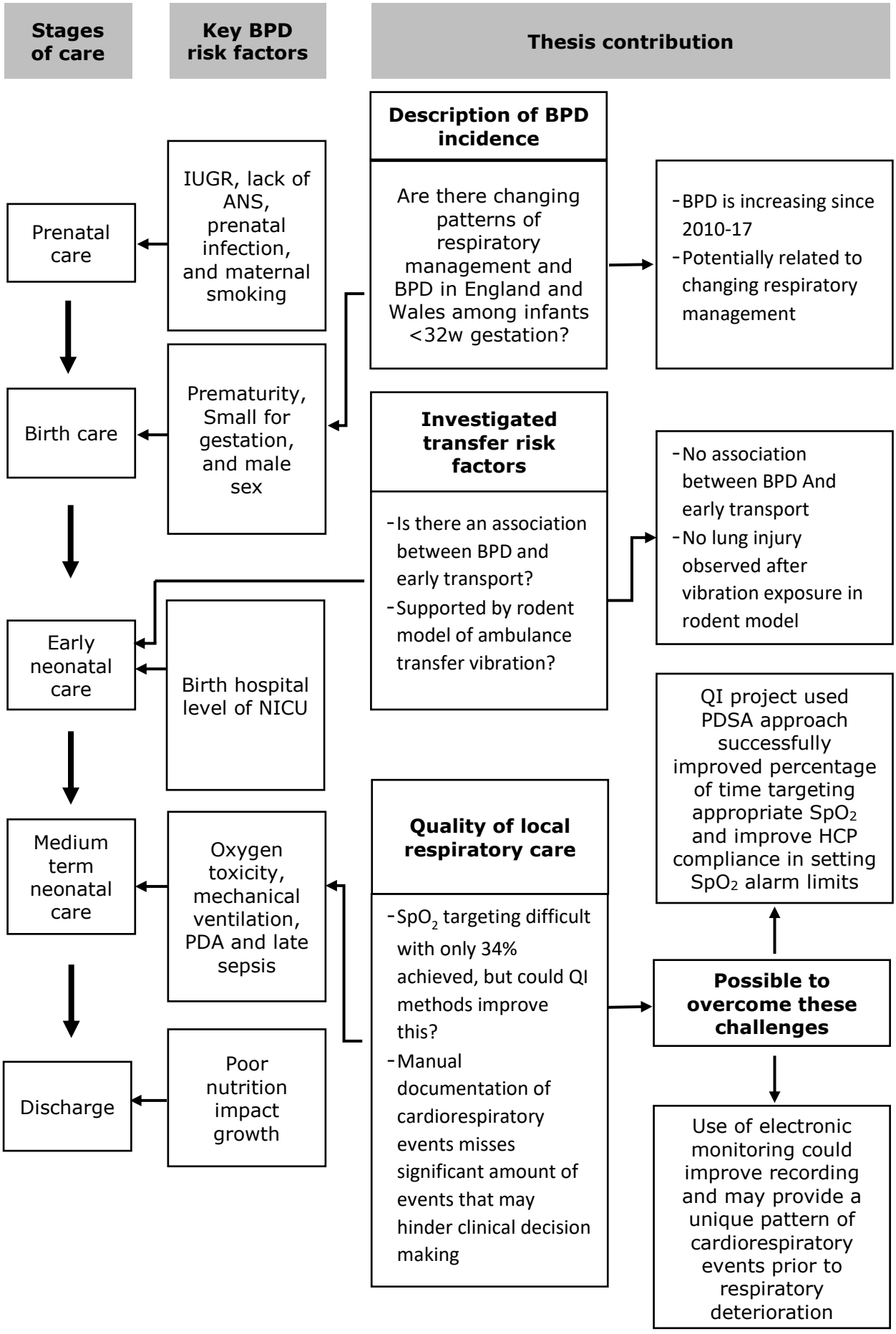


Figure 7.1 Summary of thesis contribution to the current knowledge

7.2. Summary of findings

7.2.1. Chapter 2: A national descriptive study of the temporal changes to respiratory care of infants <32 weeks gestation in England and Wales

BPD is a common respiratory disease that affects preterm infants, with poor respiratory and neurodevelopmental long-term outcomes, and it increases the health burden on the infant, their family, and healthcare resources [60, 126]. With evolving respiratory care, there was a need to understand the incidence of BPD and the current pattern of respiratory management in England and Wales during the 2010s. This could help inform future resource allocation, and identify emerging areas for research and improvement.

Chapter 2 aimed to define the incidence rate of BPD among extremely and very preterm infants born in England and Wales between 2010 and 2017, and describe the respiratory support management during the same periods. These main aims were achieved by conducting a retrospective population based cohort study using the NNRD. The main findings of this study were that the as survival improves the incidence of BPD significantly increased through the years. Furthermore, the respiratory support analysis revealed that the use of HF significantly increased over the years, whilst CPAP use declined. The lack of evidence to support these management changes could be a contributing factor in the changing rate of BPD although further, more detailed work is required to explore this further.

7.2.2. Chapter 3: Risk of BPD in extremely preterm infants transported within the first 2 days of life.

Centralisation of neonatal intensive care improves the survival of preterm infants [4]. However, it has been reported that transported infants have a higher rate of BPD compared to non-transported infants [69]. There has been little recent research about extremely preterm infant transport and subsequent development of BPD.

Chapter 3 aimed to explore the association between the incidence of BPD among preterm infants and early neonatal transport within the first 48 hours of life. In addition, this study aimed to compare the duration of MV between transported and non-transported infants before first extubation. The aims were achieved by conducting a retrospective population-based cohort study using NNRD.

The findings of this study reported that despite the increase of BPD among transported infants, the assessment of the association using multiple logistic regression models showed no association between early neonatal transport and BPD development. This was after adjusting for key factors including gestation and respiratory management. The duration of MV was found to be significantly longer among transported infants compared to non-transported ones.

7.2.3. Chapter 4: Whole body vibration during inter-hospital transfer in early neonatal life: A rat model of lung injury.

The aim of neonatal transport is to ensure safe transfer and improve the outcome for high-risk infants. However, several factors could affect this goal including the vibration encountered during transportation. Only one previous study has explored the impact of vibration on the respiratory system and found that vibration could have adverse effects, although the method used in this study might not represent what happens during real-life transport. Therefore, it was essential to undertake a more realistic study using real-life measurements to better describe the impact of vibration on the respiratory system.

Chapter 4 aimed to quantify and assess the impact of vibration, as encountered during ambulance transportation, on injury risk to the newborn lung utilising a relevant rat model. The main aim of this study was to expose rat pups to vibration and examine the mRNA expression of lung surfactant proteins, inflammatory mediators and lung pathology using a histological analysis scoring method. In this model, vibration did not induce any significant change in the mRNA expression of

surfactant proteins and important inflammatory mediators. The histological analysis found no evidence of lung injury in the studied model. This was in contrast to previous work using a rodent model that reported significant injury following vibration in parallel with ventilation.

7.2.4. Chapter 5: Targeted oxygen saturation compliance in preterm infants: Application of evidence through a local quality improvement project

Oxygen therapy is the most common drug used in neonatal units [264]. Monitoring and targeting oxygen saturations are vital to prevent complications, such as BPD, and mortality in preterm infants. One-approach used to improve targeting oxygen saturation is implementing a QI project. Several studies with different methodology have previously been conducted to improve targeting oxygen saturation, what is not clear is the optimal QI methodology to improve this goal. Therefore, this project provided a valuable opportunity to advance the understanding of implementing a local QI project to improve oxygen saturation targeting.

Chapter 5 aimed to improve clinical care by increasing the time preterm infants on oxygen therapy spent within the appropriate target oxygen saturation range. The study used a QI approach that composed of three PDSA cycles; focus on increasing HCPs awareness regarding oxygen saturations, improve the compliance of setting SpO_2 alarm limits correctly, and narrowing SpO_2 alarm limit. The findings of this study revealed that the QI approach successfully increased the percentage of time that SpO_2 kept within the appropriate target range and improved compliance of setting SpO_2 alarm limits correctly.

7.2.5. Chapter 6: Recording and utilisation of routine vital signs to predict respiratory deterioration in preterm infants

Preterm infants usually require respiratory support due to immature lung development and other factors such as sepsis [303, 304]. The use of MV is associated with lung inflammation and so avoiding the need for re-ventilation could help reduce the risk of BPD. The initiation of MV is commonly based on the HCPs experience and ability to detect clinical deterioration or the use of early warning systems. These methods are subject to a number of limitations and so could delay the detection of deterioration and possible avoidance of MV. Integration of an automated system for the care of high-risk infants could help to identify deviation from the normal physiological status and predict respiratory deterioration earlier.

Chapter 6 aimed to assess the compliance of manual documentation in recording bradycardia and desaturation events and to identify if it were possible for these events to help predict respiratory deterioration. The study aims were assessed using observational study approach. The study found the electronic monitoring system was significantly superior to traditional way of documenting bradycardia and desaturation events. A pattern of increased bradycardia and desaturation events was identified during the 24 hours before respiratory escalation occurred. With so much routinely collected vital sign data available from electronic monitors, it could be possible to train a computer system to learn about these and the patterns of individual infants. This makes personalised medicine a real potential in neonatal care, something ideally suited for infants who often spend many months in the NICU.

7.3. General strengths and limitations of the thesis

The detailed strengths and limitations were discussed in the respective chapters. However, in this section I will highlight the general strengths of the thesis and limitations that I faced while conducting my studies.

The current thesis has provided more insight into the incidence of BPD and respiratory management, aspects of investigating potential BPD risk factors related to inter-hospital transfer, and approaches to avoid BPD among preterm infants. These were accomplished using a scope of various research methodologies.

The studies contribute to the existing knowledge about the incidence of BPD among preterm infants during the last decade in England and Wales. I believe these are the most current data describing BPD and the changing patterns of respiratory support in a large dataset with almost complete population coverage.

The investigation of the association between BPD incidence and inter-hospital transfer in this thesis was explored using statistical and experimental approaches. The statistical approach was the first large contemporary population study to account for major BPD risk factors. The experimental approach investigated vibration as a risk factor for lung injury, the first to use real-life vibrational measurements for the assessment of lung injury during ambulance transportation.

The current thesis explored two different approaches that could help reduce the incidence of BPD among preterm infants. The first approach was a QI project, improving the percentage of time SpO_2 was within appropriate saturation range, although the gains were significant there was still further work needed to improve these. The second approach, an observational study, provided support for further work to define physiological vital sign patterns that could help identify impending respiratory escalation. It provides an important opportunity to advance the understanding of electronic recording systems in the NICU and possibility to develop machine-learning systems to predict respiratory deterioration in preterm infants.

The current thesis faced several limitations. The NNRD data set used in the second and third chapter did not completely cover all the neonatal units in England and Wales during the first three years (i.e. 2010 to 2012). However, coverage during this period was over 90% and it is unlikely that the characteristics of the infants and their management would be significantly different in the remaining 10% units. I planned my quality project and clinical study to use a newly developed version of OxyGUI software. Unfortunately, the optimisation of this system was not successfully attained after many months of work, reverting to the simpler original version.

The COVID-19 pandemic has disrupted my thesis writing progress in different ways. The university closure had limited the access to a quiet space and desk beside the unavailability of appropriate space to study in my accommodation, which affected my productivity.

7.4. Clinical implications of the thesis

Preterm birth is associated with significant morbidity such as BPD [61]. Consequently, preterm infants diagnosed with BPD increase the burden on public health both nationally and globally. There is a need to inform future resources regarding the rising incidence of BPD, which will increase the burden on paediatric and adult respiratory care [60, 305]. In addition, the data on respiratory support could aid the reviewing of guidelines for using HF and CPAP in neonatal settings and demonstrates the need for more studies to identify the optimal non-invasive respiratory support for preterm infants.

Through the third chapter, despite the absence of association between BPD and early life transport, it is interesting to note that the duration of MV before first extubation was significantly increased among transported infant. Given that duration of MV by itself is a major risk factor leading to BPD. The increased duration of MV in transported infants should focus clinical teams on ventilation strategies for these infants.

Through the fourth chapter, it is important to note that vibration did not induce lung injury in this model, contrary to a previous study that used MV in conjunction with vibration [85]. The concern about other factors, such as the use of excessive oxygen and MV, should be considered when planning infant transport. Importantly, the same experimental model demonstrated significant acute brain injury after vibration. Therefore, use of different techniques in minimising vibration such as changes in mattresses and transport systems could be beneficial.

Through the fifth chapter (QI project), lack of knowledge regarding SpO_2 targeting, poor compliance of setting the correct SpO_2 alarm limits and wide SpO_2 alarm limits contributed to poor SpO_2 targeting. These findings highlighted several actions that have already brought local changes at Nottingham University Hospitals such as narrowing the SpO2 alarm limit and improving compliance of setting alarms correctly. Hopefully, the implemented changes could ultimately improve the outcomes for preterm infants.

Through the sixth chapter, the findings highlighted the local weakness of manual documentation and superiority of the electronic monitoring system. Use of an electronic monitoring system could improve care by providing the clinical team with accurate data of cardiorespiratory events to aid and support their clinical decisions. In addition, the findings support the potential of developing a machine-learning algorithm that could early predict deterioration, allowing the clinical team time to intervene in the appropriate time.

7.5. Research implications of the thesis

Throughout my PhD study, several ideas have emerged as potential themes for future studies:

- As described in this thesis (chapter 2), the incidence of BPD is increasing. One of the potential causes observed is the significant increase in the use of MV among extremely preterm infants. Therefore, research studies of MV initiation, especially the compliance of the implementation of lung protective

strategies, could contribute to enhancing preterm management and ultimately reduce BPD. In addition, it was noted that the administration of surfactant replacement therapy reduced during the second epoch and this need to be investigated to understand the causes and may be linked to the increased use of MV.

- Despite little evidence found to support the use of HF versus CPAP, the use of HF over CPAP has gained significant popularity in English and Welsh neonatal settings, especially among extremely preterm infants (chapter 2). It is still not clear if the increasing use of HF is associated with rising BPD rates. Comparison of outcomes between high and low use HF centres could contribute to the growing knowledge regarding HF efficacy. This may allow new research hypotheses and subsequent well-designed trials to be initiated.
- Research is also needed to answer why the duration of MV was significantly increased among transported infant (chapter 3). Factors that could delay extubation, such as the rate of infection and use of sedative agents, should be investigated to explore potential reasons for the prolonged duration of MV among transported infant.
- Isolated vibration during transportation did not cause lung injury in the rat model (chapter 4). Modification of this study could explore the impact of MV, with same settings and duration during ambulance transportation, may provide new insights into the development of lung injury and provide a better understanding of BPD among transported infants.
- Use of QI has been shown to improve time targeting the appropriate SpO_2 saturation (chapter 5). Sustainability assessment of the improvement is recommended to assess the long-term impact of the QI. In addition, there is a need for the optimisation of QI model to include assessment of saturation targeting while infants on room air and attending list of educational sessions.

- The pattern of bradycardia and desaturation events before respiratory deterioration provides the opportunity for the development of bedside predictive monitoring systems (chapter 6). The modern age of large digital data and computing power provides an opportunity to merge large amounts of vital sign data and machine learning. An intelligent algorithm could be developed to predict earlier respiratory deterioration using routinely clinical vital sign data.

7.6. Personnel reflection

Throughout my PhD studies, I personally benefited from this opportunity to acquire new skills and develop abilities that improved me as a researcher and healthcare professional. My thesis has exposed me to a diverse range of research methodologies including a longitudinal epidemiological study, large clinical database analysis, lab-based experimental work, clinical QI project and a clinical observational study. I believe a better understanding of these methods has helped me to develop my independent research skills.

I faced challenges that I had to overcome. Examples include working with big data, lack of my previous laboratory skills, and time management for conducting all these trials within a tight timeframe.

There are great life lessons that I have learned during my PhD studies. Some of these lesions are:

- Understanding the problem in depth is key to trying to solve it.
- Good planning includes prioritising the most important tasks.
- Good time management facilitates research achievements.
- All mistakes are opportunities to learn.

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Appendices

Appendix 1: Population based ecological study - comparison of demographic characteristics for enrolled infants with detail of missing infants per characteristic (missing only reported if present).

Characteristics		Epoch1 (2010-2013) (n= 28,000)	Epoch2 (2014-2017) (n= 29,172)	P value
Intrauterine growth restriction.	No	25,663 (92)	26,313 (90)	<0.001[x]
	Yes	2,337 (8)	2,859 (10)	
Chorioamnionitis	No	27,027 (97)	27,931 (96)	<0.001[x]
	Yes	973 (3)	1,241 (4)	
Antenatal Steroid	No / incomplete	7,778 (28)	6,901 (24)	<0.001[x]
	Complete	18,714 (67)	20,784 (71)	
	missing	1,508 (5)	1,487 (5)	
Smoking in pregnancy	No	18,545 (66)	20,252 (69)	<0.001[x]
	Yes	5,084 (18)	5,115 (18)	
	missing	4,371 (16)	3,805 (13)	
Mode of delivery	vaginal	10,770 (38)	11,036 (38)	<0.001[x]
	C-section	15,367 (55)	16,662 (57)	
	missing	1,863 (7)	1,474 (5)	
Gestation in weeks, median [IQR]		29 [27-31]	29 [27-31]	0.33[u]
Gender	Male	15,177 (54)	15,791 (54)	0.86[x]
	Female	12,807 (46)	13,364 (46)	
	missing	16 (0)	17 (0)	
Apgar 1 min, median [IQR]	Recorded	7 [5-8]	6 [5-8]	<0.001[u]
	Missing, n (%)	2,794 (10)	2,792 (10)	
Apgar 5 min, median [IQR]	Recorded	9 [7-9]	9 [7-9]	<0.001[u]
	Missing, n (%)	2,946 (10)	2,901 (10)	
Birth weight g, median [IQR]		1,230 [955-1,510]	1230 [950-1,510]	0.14[u]
Surfactant	No	9,936 (35)	10,572 (36)	<0.001[x]

	Yes	17,409 (62)	16,601 (57)	
	missing	655 (2)	1,999 (7)	
≥1 course of 5 days antibiotics treatment	No	11,564 (41)	13,170 (45)	<0.001[x]
	Yes	16,436 (59)	16,002 (55)	
Surgical PDA	No	6,569 (23)	27,905 (96)	<0.001[x]
	Yes	637 (2)	373 (1)	
	missing	20,794 (74)	894 (3)	

Data presented as n(%) unless otherwise stated; n: number; IQR: interquartile range; PDA: patent ductus arteriosus
[x] indicates using of X^2 for statistical comparison; [u] indicate using of Mann-Whitney test for statistical comparison

Appendix 2: Population based ecological study - BPD incidence rate according to birth year for preterm infants below 32 weeks gestational age in England and Wales, including subgroup analysis based on gestation (i.e. extremely and very preterm infants)

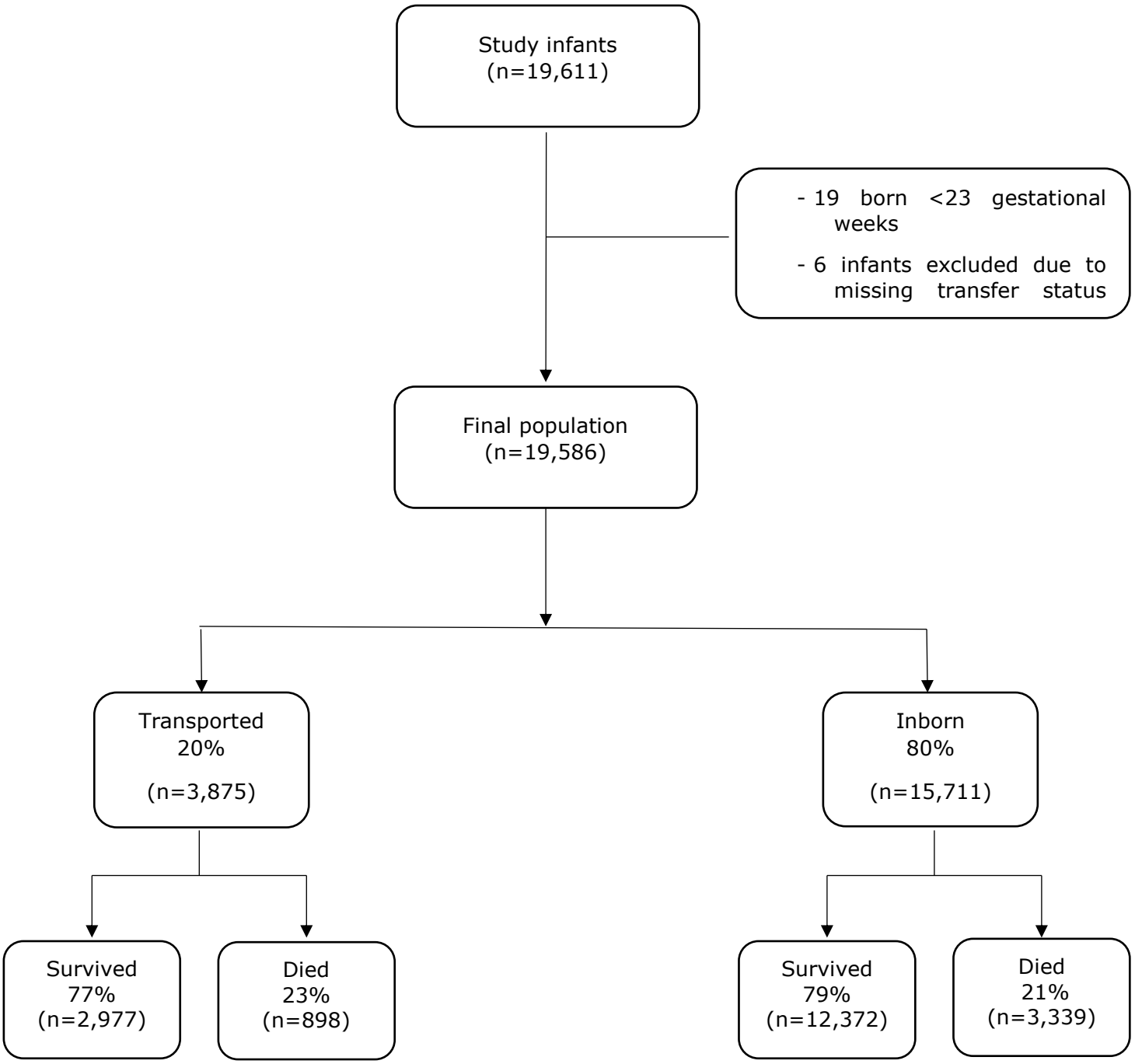
BPD outcome	Birth year								P value for X^2	P value for trend
	2010 (n=6,718)	2011 (n=7,018)	2012 (n=7,118)	2013 (n=7,146)	2014 (n=7,106)	2015 (n=7,350)	2016 (n=7,404)	2017 (n=7,312)		
all infants	1,885 (28)	2,066 (29)	2,192 (31)	2,226 (31)	2,307 (32)	2,340 (32)	2,350 (32)	2,393 (33)	<0.001	<0.001
extremely preterm infants	(n= 1,765)	(n= 1,908)	(n= 1,972)	(n= 1,939)	(n= 1,926)	(n= 2,011)	(n= 2,049)	(n= 1,973)		
	1,127 (64)	1,228 (64)	1,353 (69)	1,359 (70)	1,365 (71)	1,395 (70)	1,403 (69)	1,405 (71)	<0.001	<0.001
very preterm infants	(n= 4,953)	(n= 5,110)	(n= 5,146)	(n= 5,207)	(n=5,180)	(n=5,339)	(n=5,355)	(n=5,339)		
	758 (15)	838 (16)	839 (16)	867 (17)	942 (18)	945 (18)	947 (18)	988 (19)	<0.001	<0.001

BPD: Bronchopulmonary dysplasia; n: numbers; X2: Chi-Square test.
Data presented as number (percentage).
P values for X2 determined the significant association between mortality rate and birth year.
P value for trend determined the trend in the mortality rate across the birth year (ordinal value).

Appendix 3: Population based ecological study - the duration in days that infants below 32 weeks of gestational age spent on each respiratory support modality according to birth year.

	Respiratory support Module	Birth year							
		2010	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
All infants	MV	4 [2-11]	4 [2-12]	4 [2-12]	4 [2-11]	4 [2-11]	3 [2-11]	3 [2-11]	3 [2-11]
	CPAP	8 [3-22]	8 [3-22]	7 [2-21]	5 [2-16]	5 [2-15]	4 [2-13]	4 [2-12]	4 [2-11]
	HF	6 [2-14]	8 [3-20]	9 [3-22]	10 [4-22]	11 [4-24]	12 [4-24]	11 [4-24]	10 [4-23]
	Oxygen	13 [3.5-30]	15 [4-29]	15 [4-28]	14 [4-26]	14 [5-27]	14 [5-26]	14 [5-25]	14 [5-24]
Extremely preterm infants	MV	10 [3-27.5]	11 [4-28]	12 [4-28]	13 [4-29]	11 [4-29]	13 [4-31]	12 [4-28]	12 [4-29]
	CPAP	25 [12-38]	24 [12-37]	24 [12-36]	19 [8-32]	18 [7-30]	15 [6-27]	15 [6-27]	13 [5-25]
	HF	8 [3-18]	13 [4-27]	16 [6-29]	19 [10-31]	21 [12-33]	22 [14-32]	22 [14-33]	23 [13-35]
	Oxygen	23 [11-37]	22.5[11-35]	22 [12-35]	20 [10-30]	20 [11-30]	19 [10-29]	19 [10-29]	18 [9-28]
Very preterm infants	MV	2 [1-5]	2 [1-5]	2 [1-4]	2 [1-4]	2 [1-4]	2 [1-4]	2 [1-4]	2 [1-4]
	CPAP	5 [2-11]	4 [2-11]	4 [2-9]	3 [2-7]	3 [2-7]	3 [1-6]	3 [1-5]	2 [1-5]
	HF	5 [2-10]	5 [2-12]	6 [2-14]	6 [2-14]	6 [3-14]	6 [3-14]	6 [3-14]	6 [3-15]
	Oxygen	7 [2-22]	9 [2-22]	8 [2-22]	9 [2-21]	10 [3-23]	10 [3-22]	10 [3-21]	10 [3-21]

Appendix 4: Flowchart of the selection process of extremely preterm infants born with gestational age between 23 and 28 weeks in England and Wales during the period from 1 January 2010 to 31 December 2017 and the mortality rate for each cohort, according to transport status within the first 48 hours of life.



Appendix 5: Comparison of Gestational characteristics in infants included in assessing the composite outcome (death or BPD) according to transport status within first 48 hours of life.

Gestational Characteristics	Inborn (n=15,711)	Transported (n=3,875)	P value
Maternal Age, Mean (SD)	30.4 (6.3)	29.6 (6.2)	<0.001[t]
Missing, n (%)	123 (1)	48 (1)	
Maternal Hypertension	181(1)	23 (0.6)	0.002[x]
Preeclampsia	418 (3)	64 (2)	<0.001[x]
Maternal Diabetes	173 (1)	26 (0.7)	0.02[x]
Smoking in pregnancy	2,640 (17)	758 (20)	<0.001[x]
missing	2,478 (16)	544 (14)	
Complete course of antenatal steroid	11,118 (71)	1,693 (44)	<0.001[x]
missing	863 (5)	168 (4)	
Chorioamnionitis	1,306 (8)	107 (3)	<0.001[x]
Intrauterine Growth Restriction	901 (6)	102 (3)	<0.001[x]
C-section delivery	6,166 (39)	1,307 (34)	<0.001[x]
missing	967 (6)	157 (4)	

Data presented as n(%) unless otherwise stated; n: number; SD: standard deviation; C-section: caesarean section

[x] indicates using of Chi squared test for statistical comparison; [t] indicates using of independent t-test for statistical comparison.

Appendix 6: Comparison of neonatal characteristics in infants <28 weeks included in assessing the composite outcome (death or BPD) according to transport status within first 48 hours of life.

Neonatal Characteristics	Inborn (n=15,711)	Transported (n=3,875)	P Value
Gestational age in weeks, mean(SD)	25.57(1.3)	25.33(1.2)	<0.001[t]
Birthweight in g, mean(SD)	829 (196)	827 (180)	0.63[t]
Male	8,503 (54)	2,161(56)	0.06[x]
Apgar Score 1 min, median (IQR)	5 (3-7)	5 (3-6)	<0.001[u]
Missing	1,896 (12)	431 (11)	
Apgar Score 5 min, median (IQR)	8 (6-9)	7 (6-9)	<0.001[u]
Missing	1,975 (13)	460 (12)	
Multiple pregnancy	3,998 (25)	894 (23)	0.002[x]
missing	4 (0.03)	1 (0.03)	
Necrotising enterocolitis	3,085 (20)	895 (23)	0.01[x]
missing	3,514 (22)	611 (16)	
Infants received at least one episode of (5 days antibiotic treatment)	11,692 (74)	3,041 (78)	<0.001[x]

Data presented as n (%) unless otherwise stated; n: number; IQR: interquartile range; SD: standard deviation.
[x] indicates using of Chi squared test for statistical comparison; [t] indicates using of independent t-test for statistical comparison; [u] indicate using of Mann-Whitney test for statistical comparison.

Appendix 7: Comparison of infant respiratory management factors in infants <28 weeks included in assessing the composite outcome (death or BPD) according to transport status within first 48 hours of life.

Respiratory management	Inborn (n=15,711)	Transported (n=3,875)	P Value
Number of infants on MV	14,962 (95)	3,847 (99)	<0.001[x]
Duration of MV, Median (IQR)	10 (3-26)	12 (5-28)	<0.001[u]
Number of infants on oxygen therapy	10,998 (70)	2,667 (69)	0.15[x]
Duration of oxygen therapy, Median (IQR)	20 (10-32)	20 (10-31)	0.79[u]
Number of infants on HF	9,478 (60)	2,366 (61)	0.40[x]
Duration of days on HF, Median (IQR)	19 (10-32)	20 (10-31)	0.78[u]
Number of infants on CPAP	11,707 (74.5)	2,972 (77)	0.005[x]
Duration of days on CPAP, Median (IQR)	18 (7-31)	19 (8-31)	0.01[u]

Data presented as n(%) unless otherwise stated; n: number; MV: mechanical ventilation ; CPAP: continuous positive airway pressure ; HF: high flow; IQR: interquartile range
[x] indicated using of chi-squared test ; U indicate using of Mann-Whitney test for statistical comparisons

Appendix 8: Comparison of gestational characteristics of preterm infant born between 23 and 25 weeks of gestational age including in assessing the composite outcome (death or BPD) according to transport status within the first 48 hours of life.

Gestational Characteristics	**Group1: 23 - 25 gestational weeks (n=8,726)**		
	Inborn (n=6,722)	**Transported (n=2,004)**	**P value**
Maternal Age, Mean (SD)	30.3 (6.3)	29.6 (6.2)	<0.0001[t]
Missing, n (%)	66 (1)	22 (1)	
Maternal Hypertension	60 (1)	10 (0.5)	0.08[x]
Preeclampsia	89 (1)	15 (1)	0.04[x]
Maternal Diabetes	66 (1)	13 (1)	0.17[x]
Smoking in pregnancy	1,103 (16)	373 (19)	0.06[x]
missing	1,064 (16)	274 (14)	
Complete course of antenatal steroid	4,662 (69)	797 (40)	<0.001[x]
missing	380 (6)	97 (5)	
Chorioamnionitis	716 (11)	67 (3)	<0.001[x]
Intrauterine Growth Restriction	160 (2)	27 (1)	0.005[x]
Multiple Pregnancy	1,765 (26)	419 (21)	<0.001[x]
C-section delivery	1,437 (21)	418 (21)	0.31[x]
missing	421 (6)	72 (4)	

Data presented as n(%) unless otherwise stated; GA: gestational age; n: number; SD: standard deviation; C-section: caesarean section
[x] indicates using of Chi squared test for statistical comparison; [t] indicates using of independent t-test for statistical comparison.

Appendix 9: Comparison of neonatal characteristics of preterm infant born between 23 and 25 weeks of gestational age including in assessing the composite outcome (death or BPD) according to transport status within the first 48 hours of life.

Neonatal Characteristics	Group1: 23 - 25 gestational weeks (n=8,726)		
	Inborn n=6,722	**Transported n=2,004**	**P Value**
Gestational age in weeks, mean(SD)	24.2 (0.7)	24.3 (0.7)	0.002[t]
Birthweight in g, mean(SD)	692 (121)	719 (119)	<0.001[t]
Male	3,606 (54)	1,104 (55)	0.25[x]
Apgar Score 1 min, median (IQR)	4 (2-6)	4 (2-6)	0.54[u]
Missing	872 (13)	234 (12)	
Apgar Score 5 min, median (IQR)	7 (5-8)	7 (5-8)	0.04[u]
Missing	880 (13)	248 (12)	
Surfactant	6,356 (95)	1,967 (98)	<0.001[x]
missing	242 (4)	22 (1)	
Necrotising enterocolitis	1,494 (22)	482 (24)	0.62[x]
missing	1,406 (21)	326 (16)	
Infants received at least one episode of (5 days	5,090 (76)	1,588 (79)	0.001[x]
Surgical PDA	526 (8)	151 (7.5)	0.41[x]
missing	2,291 (34)	638 (32)	

Data presented as n (%) unless otherwise stated; n: number; IQR: interquartile range; SD: standard deviation.
[x] indicates using of Chi squared test for statistical comparison; [t] indicates using of independent t-test for statistical comparison; [u] indicate using of Mann-Whitney test for statistical comparison.

Appendix 10: Comparison of respiratory management factors of preterm infant born between 23 and 25 weeks of gestational age including in assessing the composite outcome (death or BPD) according to transport status within the first 48 hours of life.

respiratory management	Group1: 23 - 25 gestational weeks (n=8,726)		
	Inborn (n=6,722)	**Transported (n=2,004)**	**P Value**
Number of infants on MV	6,596 (98)	2,003 (100)	<0.001[x]
Duration of MV, Median (IQR)	20 (7-37)	20 (8-36)	0.08[u]
Number of infants on oxygen therapy	4,096 (61)	1,202 (60)	0.44[x]
Duration of oxygen therapy, Median (IQR)	22 (12-33)	21 (11-33)	0.17[u]
Number of infants on HF	3,622 (54)	1,078 (54)	0.94[x]
Duration of days on HF, Median (IQR)	22 (12-35)	22 (12-35)	0.53[u]
Number of infants on CPAP	4,464 (66)	1,365 (68)	0.15[x]
Duration of days on CPAP, Median (IQR)	24 (11-36)	23 (12-35)	0.36[u]

Data presented as n(%) unless otherwise stated; n: number; MV: mechanical ventilation ; CPAP: continuous positive airway pressure ; HF: high flow; IQR: interquartile range
[x] indicated using of chi-squared test ; U indicate using of Mann-Whitney test for statistical comparisons

Appendix 11: Comparison of gestational characteristics of preterm infant born at 26 and 27 weeks of gestational age including in assessing the composite outcome (death or BPD) according to transport status within the first 48 hours of life.

Gestational Characteristics	Group 2: 26 - 27 gestational weeks (n=10,860)		
	Inborn n=8,989	transported n=1,871	P value
Maternal Age, Mean (SD)	30.4 (6.3)	29.6 (6.3)	<0.0001[t]
Missing	57 (1)	26 (1)	
Maternal Hypertension	121 (1)	13 (1)	0.02[x]
Preeclampsia	329 (4)	49 (3)	0.02[x]
Maternal Diabetes	107 (1)	13 (1)	0.06[x]
Smoking in pregnancy	1,537 (17)	385 (21)	0.001[x]
missing	1,414 (16)	270 (14)	
Complete course of antenatal steroid	6,456 (72)	896 (48)	<0.001[x]
missing	483 (5)	71 (4)	
Chorioamnionitis	590 (7)	40 (2)	<0.001[x]
Intrauterine Growth Restriction	741 (8)	75 (4)	<0.001[x]
Multiple Pregnancy	2,233 (30)	475 (25)	0.61[x]
C-section delivery	4,729 (53)	889 (47.5)	<0.001[x]
missing	546 (6)	85 (4.5)	

Data presented as n (%) unless otherwise stated; n: number; SD: standard deviation; C-section: caesarean section

[x] indicates using of Chi square test for statistical comparison; [t] indicates using of independent t-test for statistical comparison.

Appendix 12: Comparison of neonatal characteristics of preterm infant born at 26 and 27 weeks of gestational age including in assessing the composite outcome (death or BPD) according to transport status within the first 48 hours of life.

Neonatal Characteristics	Group 2: 26 - 27 gestational weeks (n=10,860)		
	Inborn n=8,989	**Transported n=1,871**	**P Value**
Gestational age in weeks, mean(SD)	26.6 (0.5)	26.4 (0.5)	<0.001[t]
Birthweight in g, mean(SD)	931 (178)	943 (162)	0.01[t]
Male	4,897 (54)	1,057 (56)	0.11[x]
Apgar Score 1 min, median (IQR)	5 (4-7)	5 (3-7)	<0.001[u]
Missing	1,024 (11)	197 (10.5)	
Apgar Score 5 min, median (IQR)	8 (7-9)	8 (6-9)	<0.001[u]
Missing	1,095 (12)	212 (11)	
Surfactant	7,996 (89)	1,801 (96)	<0.001[t]
missing	336 (4)	32 (2)	
Necrotising enterocolitis	1,591 (18)	413 (22)	0.01[t]
missing	2,108 (23)	285 (15)	
Infants received at least one episode of (5 days	6,602 (73)	1,453 (78)	<0.001[t]
Surgical PDA	226 (2.5)	45 (2.4)	0.3[t]
missing	3,519 (39)	587 (31)	

Data presented as n (%) unless otherwise stated; n: number; IQR: interquartile range; SD: standard deviation.
[x] indicates using of Chi squared test for statistical comparison; [t] indicates using of independent t-test for statistical comparison; [u] indicate using of Mann-Whitney test for statistical comparison.

Appendix 13: Comparison of respiratory management factors of preterm infant born at 26 and 27 weeks of gestational age including in assessing the composite outcome (death or BPD) according to transport status within the first 48 hours of life.

Infants respiratory management factors	Group 2: 26 - 27 gestational weeks (n=10,860)		
	Inborn (n=8,989)	Transported (n=1,871)	P Value
Number of infants on MV	8,366 (93)	1,844 (99)	<0.001[x]
Duration of MV, Median (IQR)	6 [2-14]	8 [4-16]	<0.0001[u]
Number of infants on HF	5,856 (65)	1,288 (69)	0.002[x]
Duration of days on HF, Median (IQR)	18 [9-29]	18 [9-28]	0.95[u]
Number of infants on CPAP	7,243 (81)	1,607 (86)	<0.001[x]
Duration of days on CPAP, Median (IQR)	14 [5-27]	15 [6-27]	0.09[u]
Number of infants on oxygen therapy without pressure support	6,902 (77)	1,465 (78)	0.16[x]
Duration of oxygen therapy without pressure support, Median (IQR)	19 [9-30]	19 [9-30]	0.99[u]

Data presented as n(%) unless otherwise stated; n: number; MV: mechanical ventilation ; CPAP: continuous positive airway pressure ; HF: high flow; IQR: interquartile range
[x] indicated using of chi-squared test ; U indicate using of Mann-Whitney test for statistical comparisons

Appendix 14: Association of transportation with BPD by complete cohorts and gestational subgroups (sensitivity analysis adjusted for all BPD risk factors)

Cohort	OR (95% CI)	Adjusted OR (95% CI)[a]
All infants (n=15,543)	1.16 (1.06-1.26)	1.03 (0.85-1.25)
23-25 weeks (n=5,918)	0.81 (0.69-0.95)	0.86 (0.63-1.18)
26-27 weeks (n=9,625)	1.18 (1.06-1.31)	1.11 (0.87-1.42)

n: number; OR: odd ratio; CI: confidence interval

[a] adjusted for gender, gestational age in weeks, birth weight, maternal smoking in pregnancy, complete course of antenatal steroids, diagnose of chorioamnionitis, diagnose of intrauterine growth restriction, diagnose of preeclampsia, multiple pregnancy, mode of delivery, Apgar score in 1 and 5 minutes, surfactant therapy, infants received at least 1 episode of 5 days antibiotic treatments, diagnose of PDA that required surgical therapy, receive of at least one day respiratory support via mechanical ventilation (MV), duration of MV, receive of at least one day respiratory support via oxygen therapy, duration of oxygen therapy, receive of at least one day respiratory support via high flow (HF), duration of HF, receive of at least one day respiratory support via continuous positive airway pressure (CPAP), duration of CPAP.

Appendix 15: List of Suppliers for lab experimental work.

Grant-bio	Cambridge, United Kingdom https://www.grantinstruments.com/
Dytran	California, Unites States https://www.dytran.com/
Svantek	Warsaw, Poland https://www.svantek.com/
Ambion	Loughborough, UK https://www.thermofisher.com/uk/en/home.html
Sigma-Aldrich	Gillingham, United Kingdom https://www.sigmaaldrich.com/united-kingdom.html
Medic Tools	Zurich, Switzerland
Fisher Scientific	Loughborough, United Kingdom https://www.fishersci.co.uk/gb/en/home.html
Qiagen	Manchester, United Kingdom https://www.qiagen.com/us/default.aspx
NanoDrop Technologies	Loughborough, UK https://www.thermofisher.com/uk/en/home.html
Applied Biosystems	Loughborough, UK https://www.thermofisher.com/uk/en/home.html
TechEn	Massachusetts, United States https://www.techen.com/
Primer design Ltd	Chandler's Ford, United Kingdom http://www.primerdesign.co.uk/home
BioRad	California, United States https://www.bio-rad.com/
Abgene	Loughborough, UK https://www.thermofisher.com/uk/en/home.html
Applied Biosciences	Loughborough, UK https://www.thermofisher.com/uk/en/home.html
Simport	Quebec, Canada http://www.simport.com/en/

Anglia	Wisbech, United Kingdom https://www.anglia.com/
Thermo Fisher	Loughborough, UK https://www.thermofisher.com/uk/en/home.html
Thermo Scientific	Loughborough, UK https://www.thermofisher.com/uk/en/home.html
VWR	Lutterworth, United Kingdom https://uk.vwr.com/store/
Nikon	Surrey, United Kingdom https://www.nikon.com/
Perkin Elmer	Seer Green, United Kingdom https://www.perkinelmer.com/uk/
QImaging	Surrey, Canada https://www.qimaging.com/
Microsoft Corporation	Berkshire, United Kingdom https://www.microsoft.com/
GraphPad Prism	California, United States https://www.graphpad.com/
Minitab	Coventry, United Kingdom https://www.minitab.com/

Appendix 16: Average result of nanodrop spectrophotometer 260/280 optical density ratio (two per sample). C represents control, V represents vibration. M represents male and F represents female.

Postnatal day 7 samples				**Postnatal day 4 samples**			
Sample	Group	Gender	260/280	**Sample**	Group	Gender	260/280
101	C	F	2.09	**701**	C	F	2.12
102	C	F	2.05	**702**	V	M	2.10
103	V	M	2.175	**703**	C	M	2.10
104	V	M	2.17	**704**	C	F	2.12
201	C	M	2.17	**705**	C	M	2.10
202	C	M	2.18	**706**	V	F	2.12
203	V	F	2.10	**707**	V	F	2.11
204	V	F	2.11	**708**	V	M	2.1
301	C	F	2.11	**709**	C	F	2.06
302	C	F	2.11	**710**	V	F	2.11
303	V	F	2.13	**801**	C	M	2.13
304	V	F	2.12	**802**	C	M	2.1
305	C	M	2.17	**803**	V	F	2.11
306	C	M	2.17	**804**	C	F	2.08
401	C	F	2.11	**805**	V	M	2.07
403	C	F	2.12	**806**	C	F	2.11
404	V	M	2.17	**807**	V	F	2.11
405	V	M	2.14	**808**	V	M	2.09
501	V	F	2.11	**809**	C	M	2.11
502	V	F	2.12	**810**	V	F	2.11
503	C	M	2.14	**811**	C	F	2.08
504	C	M	2.13	**812**	C	F	2.12
505	V	M	2.15	**813**	V	M	2.11
601	C	F	2.12	**814**	V	F	2.1
602	V	F	2.13	**815**	V	M	2.12
603	V	F	2.12				
604	V	M	2.13				
605	V	M	2.14				

Appendix 17: Lab experimental work - primePCR assay validation reports obtained from the manufacture (BioRad).

Surfactant Protein A

Gene Symbol	SPA
Organism	Rat
UniGene ID	Rn.160052
Unique Assay ID	qRnoCEP0031932
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000074460
Amplicon Context Sequence	GGGCCTGCTAGGTCCAAGGCATGCTCACTCCCCAAAGGCTGAC CGCTCCAGGC CCACGCAGGCTTCAGCTGCTCTCAGGCTCTGCAGGTAAGGTGG TGTGGTGGCC CTTTATGATGAAGGGTGACATTGGTCCCCGGTGAGAGG
Amplicon Length (bp)	114
Chromosome Location	20:5701932-5702075
Assay Design	Exonic
Purification	Desalted
Efficiency (%)	98
R^2	0.9994
cDNA Cq	28.41
cDNA Tm (Celsius)	87.5
gDNA Cq	25.09
Specificity (%)	100

Surfactant Protein B

Gene Symbol	SPB
Organism	Rat
UniGene ID	Rn.1952
Unique Assay ID	qRnoCEP0031431
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000014505
Amplicon Context Sequence	TGCCTGGCTGAGCGTTACACAGTACTTCTACTAGATGCAC TGCTGGGTCGTGTG GTGCCCCAGCTAGTCTGTGGCCTGGTCCTCCGATGTTCCA CTGCAGATGCCATT GGCCCAGCCCTCCCTGCT
Amplicon Length (bp)	96
Chromosome Location	4:164942126-164942905
Assay Design	Exonic
Purification	Desalted
Efficiency (%)	101
R^2	0.9993
cDNA Cq	Target not expressed in universal RNA
cDNA Tm (Celsius)	Target not expressed in universal RNA
gDNA Cq	Target not expressed in universal RNA
Specificity (%)	No cross reactivity detected

Surfactant Protein C

Gene Symbol	SPC
Organism	Rat
UniGene ID	Rn.3926
Unique Assay ID	qRnoCIP0025408
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000015035
Amplicon Context Sequence	AATCACCACGACGACAAGGACTACCACCACAACCACGATGA GAAGGCGTTTGAG ATGCACGGGGCAGCAGGGAATGCGAAACTGGCTCCTGGGA CCTGTCGAGTAAT CCGGTGGGCTCTCCATCAGTACCTCTTTGCTACCCATGTC
Amplicon Length (bp)	117
Chromosome Location	15:55936707-55937983
Assay Design	Intron-spanning
Purification	Desalted
Efficiency (%)	102
R^2	0.9978
cDNA Cq	34.77
cDNA Tm (Celsius)	86
gDNA Cq	43.95
Specificity (%)	100

Surfactant Protein D

Gene Symbol	SPD
Organism	Rat
UniGene ID	Rn.11348
Unique Assay ID	qRnoCIP0031383
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000074559
Amplicon Context Sequence	CTGGACCTCTAGGGCCAGGCAACCCTGAGAGTCCCATAG GTCCTGGCAAACCT GGATCACCCTTCTCGCCCCGTGGACCCTCTCTGCCATCCC GTCCATCACGACCA GGCAGGCCATTCTCTGTTGGACTACACAAGACTAGGGTGCAT
Amplicon Length (bp)	119
Chromosome Location	16:18626201-18631448
Assay Design	Intron-spanning
Purification	Desalted
Efficiency (%)	101
R^2	0.9997
cDNA Cq	32.25
cDNA Tm (Celsius)	86.5
gDNA Cq	41.07
Specificity (%)	100

Toll like receptor 4

Gene Symbol	TLR4
Organism	Rat
UniGene ID	Rn.14534
Unique Assay ID	qRnoCEP0024776
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000014020
Amplicon Context Sequence	CATGGGTCTAGAAGAGCTGGAATACCTGGACTTTCAGCACT CCACTTTAAAAAAG GTCACAGAATTCTCAGTGTTCTTATCTCTTGAAAAACTTCTT TACCTTGACATCTC TTACACTAATACCAAAATTGACTTTGATGGCATATT
Amplicon Length (bp)	117
Chromosome Location	5:86702446-86702592
Assay Design	Exonic
Purification	Desalted
Efficiency (%)	102
R^2	0.9994
cDNA Cq	22.46
cDNA Tm (Celsius)	79
gDNA Cq	
Specificity (%)	100

Nuclear factor kappa B

Gene Symbol	NFKB
Organism	Rat
UniGene ID	Rn.2411
Unique Assay ID	qRnoCIP0025529
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000042316, ENSRNOT00000036838, ENSRNOT00000040511, ENSRNOT00000068674
Amplicon Context Sequence	TCAGAATCTCCCTGTCGTCACTCTTGGCACAATCTCTAGGCT CGTTTTTAAATTTG GTGTTTATGGTGCCATGGGTGATGCCTGTGTTGGATTTAG TGGCTCCGGGATGG AATGCAATCCC
Amplicon Length (bp)	91
Chromosome Location	2:259350292-259351746
Assay Design	Intron-spanning
Purification	Desalted
Efficiency (%)	92
R^2	0.9979
cDNA Cq	22.58
cDNA Tm (Celsius)	81.5
gDNA Cq	42.23
Specificity (%)	100

Tumour necrosis factor α

Gene Symbol	TNFα
Organism	Rat
UniGene ID	Rn.2275
Unique Assay ID	qRnoCEP0030948
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000001110
Amplicon Context Sequence	CTACTTTGGAGTCATTGCTCTGTGAGGCGACTGGCGTGTTC ATCCGTTCTCTACC CAGCCCCTGTCCCCGACTCTGACCCCCATTACTCTGACCCC TTTATCGTCTACTC CTCAGAGCCCCCAATCTGTGTCCTTCTAACTT
Amplicon Length (bp)	112
Chromosome Location	20:6937988-6938129
Assay Design	Exonic
Purification	Desalted
Efficiency (%)	98
R^2	0.9997
cDNA Cq	26.55
cDNA Tm (Celsius)	85
gDNA Cq	25.57
Specificity (%)	100

Interleukin 1 β

Gene Symbol	IL1β
Organism	Rat
UniGene ID	Rn.9869
Unique Assay ID	qRnoCIP0026511
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000006308
Amplicon Context Sequence	TGAAAGCTCTCCACCTCAATGGACAGAACATAAGCCAACAA GTGGTATTCTCCAT GAGCTTTGTACAAGGAGAGACAAGCAACGACAAAATCCCT GTGGCCTTGGGCCT CAAGGGGAAGAATCTATACCTGTCCTGTGTGATGAAAGACG
Amplicon Length (bp)	120
Chromosome Location	3:128137648-128138943
Assay Design	Intron-spanning
Purification	Desalted
Efficiency (%)	98
R^2	0.9991
cDNA Cq	25.95
cDNA Tm (Celsius)	82.5
gDNA Cq	
Specificity (%)	100

Monocyte Chemoattractant Protein 1

Gene Symbol	MCP1
Organism	Rat
UniGene ID	Rn.4772
Unique Assay ID	qRnoCEP0031103
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000009448
Amplicon Context Sequence	TAAATCTGAAGCTAATGCATCCACTCTCTTTTCCACAACCAC CTCAAGCACTTCTG TAGAAGTGACCAGTATGACAGAGAACTAGTGTGATTTGGAA TGTGATGCCTTAAG TAATGTTAAACTTATTTAACT
Amplicon Length (bp)	102
Chromosome Location	10:69048547-69048678
Assay Design	Exonic
Purification	Desalted
Efficiency (%)	94
R^2	0.9989
cDNA Cq	18.91
cDNA Tm (Celsius)	80.5
gDNA Cq	25.28
Specificity (%)	100

Transforming growth factor β 1

Gene Symbol	TGFβ1
Organism	Rat
UniGene ID	Rn.40136
Unique Assay ID	qRnoCIP0031022
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000028051
Amplicon Context Sequence	TTTTGACGTCACTGGAGTTGTCCGGCAGTGGCTGAACCAA GGAGACGGAATACA GGGCTTTCGCTTCAGTGCTCACTGCTCTTGTGACAGCAAAG ATAATGTACTCCAC GTGGAAATCAATGGGATCAGTCCCAAACGT
Amplicon Length (bp)	109
Chromosome Location	1:83747870-83750404
Assay Design	Intron-spanning
Purification	Desalted
Efficiency (%)	99
R^2	0.9996
cDNA Cq	19.24
cDNA Tm (Celsius)	84
gDNA Cq	
Specificity (%)	100

Ribosomal protein L13a

Gene Symbol	RPL13a
Organism	Rat
UniGene ID	Rn.92211
Unique Assay ID	qRnoCEP0050813
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000027976
Amplicon Context Sequence	GGCACAAACAGTCTTTATTGGGTTCACACCAAGAGTCCATT GGTCTTGAGGACCT CTGTGAACTTGCAGATTTTCTTCTCCACATTCTTTTCTGCCT GTTTCCTTAGCCTC AAGAGC
Amplicon Length (bp)	87
Chromosome Location	1:102186223-102186424
Assay Design	Exonic
Purification	Desalted
Efficiency (%)	94
R^2	0.9998
cDNA Cq	15.3565
cDNA Tm (Celsius)	80
gDNA Cq	22.2693
Specificity (%)	

Ribosomal protein S29

Gene Symbol	RPS29
Organism	Rat
UniGene ID	Rn.34942
Unique Assay ID	qRnoCIP0049380
Assay Type	Probe
Detected Coding Transcript	ENSRNOT00000005577, ENSRNOT00000041384, ENSRNOT00000050726, ENSRNOT00000051764
Amplicon Context Sequence	TTTATTCTGTGTGCACAAAGACTAGCATGATTGGTATCACA GGGTAGACAGTTGG TTTCATTGGGTAGACAGTCGAATCATCCATTCAGGTCGCTT AGTCCAACTTAATG AAGCCTATGTCCTTCGCGTACTGACGG
Amplicon Length (bp)	107
Chromosome Location	6:100914122-100915088
Assay Design	Intron-spanning
Purification	Desalted
Efficiency (%)	84
R^2	0.992
cDNA Cq	14.289
cDNA Tm (Celsius)	80.5
gDNA Cq	21.8446
Specificity (%)	99.89

Appendix 18: Summary of normalised mRNA expression comparing control and intervention group at postnatal day 4 & 7

Gene	D4 Mod C (n=5)	D4 Mod V (n=6)	P value	D4 High C (n=7)	D4 High V (n=7)	P value	D7 Mod C (n=6)	D7 Mod V (n=7)	P value	D7 High C (n=8)	D7 High V (n=8)	P value
SPA	1.3 ± 0.1	1.3 ± 0.1	0.79	1.1 ± 0.1	1.2 ± 0.1	0.42	0.9 ± 0.1	1.0 ± 0.1	0.59	0.7 ± 0.1	0.8 ± 0.1	0.47
SPB	0.9± 0.2	1.5 ± 0.2	0.11	0.8 ± 0.1	0.9 ± 0.1	0.32	1.0 ± 0.2	1.1 ± 0.2	0.66	0.9 ± 0.2	1.0 ± 0.2	0.70
SPC	1.3 ± 0.2	1.3 ± 0.1	0.95	0.9 ± 0.0	1.0 ± 0.1	0.39	0.8 ± 0.1	0.8 ± 0.1	0.65	0.9 ± 0.2	0.9± 0.2	0.84
SPD	1.0 ± 0.2	1.2 ± 0.1	0.34	0.8 ± 0.1	1.0 ± 0.1	0.23	0.9 ± 0.2	1.0 ± 0.1	0.72	0.8 ± 0.1	0.7 ±0.1	0.38
NF-Kβ	1.0 ± 0.1	0.9 ± 0.1	0.71	1.0 ± 0.1	1.2 ± 0.1	0.09	1.1 ± 0.2	1.1 ± 0.1	0.74	1.4 ± 0.1	1.2 ± 0.1	0.16
TLR4	1.2 ±0.2	2.0 ±0.9	0.93	1.2 ± 0.1	1.4 ± 0.1	0.24	0.9 ± 0.1	0.9 ± 0.1	1.00	1.1 ± 0.1	0.8 ± 0.1	0.05
TGFβ1	1.0 ± 0.2	1.0 ± 0.1	0.96	1.0 ± 0.0	1.2 ± 0.1	0.27	1.2 ± 0.1	1.1± 0.1	0.78	1.2 ± 0.1	1.0 ± 0.1	0.40
MCP1	1.1 ± 0.1	0.8 ± 0.1	0.19	1.1 ± 0.2	1.1 ± 0.1	0.92	0.4 ± 0.0	0.8 ± 0.2	0.05	0.9 ± 0.2	0.7 ± 0.1	0.28
IL1β	1.1 ± 0.1	0.8 ± 0.1	0.08	1.2 ± 0.1	1.1 ± 0.1	0.52	1.0 ± 0.1	1.0 ± 0.1	0.90	0.8 ± 0.1	0.8± 0.1	0.93
TNFα	1.0 ± 0.1	0.9 ± 0.1	0.74	1.0 ± 0.1	1.0 ± 0.1	0.73	0.6 ± 0.1	1.1 ± 0.4	0.73	0.7 ±0.2	0.5 ±0.1	0.44

In the table, C: control, V: vibration, Mod: moderate level, High: High level. Values are mean ± standard error of mean. The Statistical analysis: un-paired t test used for normally distributed data, and the Mann-Whitney test for non-parametric data

Appendix 19: NeoSat QI - healthcare Professional's survey

Q1. Which neonatal unit do you mainly work on?

- City hospital
- QMC

Q2. What is your role?

- Doctor
- Nurse
- ANNP

Q3. How many years have you been qualified in your role?

- Less than 1 year
- 1-3 years
- 4-8 years
- More than 8 years

Q4. Do you know if your unit has a written policy or guideline regarding oxygen targeting range for neonates?

- Yes
- No
- Unsure

Q5 to Q9 related to optimal oxygen saturation targeting

Q5. What is the optimal UPPER target oxygen saturation in an infant <33 weeks gestation in oxygen on the first day of life?

• 100%	• 95%	• 90%	• 85%
• 99%	• 94%	• 89%	• <85%
• 98%	• 93%	• 88%	
• 97%	• 92%	• 87%	
• 96%	• 91%	• 86%	

Q6. What is the optimal LOWER target oxygen saturation in an infant <33 weeks gestation on the first day of life?

• 100%	• 95%	• 90%	• 85%
• 99%	• 94%	• 89%	• <85%
• 98%	• 93%	• 88%	
• 97%	• 92%	• 87%	
• 96%	• 91%	• 86%	

Q7. For the preterm infant <33 weeks gestation in oxygen, which of the following conditions are associated with oxygen saturations frequently BELOW YOUR LOWER target (select all that apply)?

- Chronic lung disease (bronchopulmonary dysplasia)
- Necrotising enterocolitis (NEC)
- Retinopathy of prematurity (ROP)
- Late onset infection
- Intraventricular haemorrhage (IVH)
- Death
- No risk at all

Q8. For the preterm infant <33 weeks gestation in oxygen, which of the following conditions are associated with oxygen saturations frequently OVER YOUR UPPER target (select all that apply)?

- Chronic lung disease (bronchopulmonary dysplasia)
- Necrotising enter colitis (NEC)
- Retinopathy of prematurity (ROP)
- Late onset infection
- Intraventricular haemorrhage (IVH)
- Death
- No risk at all

Q9. For any preterm infant <33 weeks gestation in air during the first day of life, what is the lower oxygen saturation alarm limit appropriate for monitoring?

• 100 %	• 95 %	• 90 %	• 85 %	• 80 %	• 75%
• 99%	• 94 %	• 89 %	• 84 %	• 79 %	• 74%
• 98%	• 93 %	• 88 %	• 83 %	• 78 %	• 73%
• 97%	• 92 %	• 87 %	• 82 %	• 77 %	• 72%
• 96%	• 91 %	• 86 %	• 81 %	• 76 %	• 71%
					• 70%
					• <70 %

Q10 and Q11 related to oxygen saturation alarm limits

Q10. For any preterm infant <33 weeks gestation in oxygen during the first day of life, what is the UPPER oxygen saturation alarm limit appropriate for monitoring?

• 100 %	• 95 %	• 90 %	• 85 %	• 80 %	• 75%
• 99%	• 94 %	• 89 %	• 84 %	• 79 %	• 74%
• 98%	• 93 %	• 88 %	• 83 %	• 78 %	• 73%
• 97%	• 92 %	• 87 %	• 82 %	• 77 %	• 72%
• 96%	• 91 %	• 86 %	• 81 %	• 76 %	• 71%
					• 70%
					• <70 %

Q11. For any preterm infant <33 weeks gestation in oxygen during the first day of life, what is the LOWER oxygen saturation alarm limit appropriate for monitoring?

• 100 %	• 95 %	• 90 %	• 85 %	• 80 %	• 75%
• 99%	• 94 %	• 89 %	• 84 %	• 79 %	• 74%
• 98%	• 93 %	• 88 %	• 83 %	• 78 %	• 73%
• 97%	• 92 %	• 87 %	• 82 %	• 77 %	• 72%
• 96%	• 91 %	• 86 %	• 81 %	• 76 %	• 71%
					• 70%
					• <70 %

Q12. Please select one response for each section on the statement: Targeting of oxygen saturations in preterm infants is challenging because: *(this question included only in pre-survey)*

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
The alarm limits are too wide					
The alarm limits are too narrow					
Caring for multiple babies makes it hard to respond to saturations outside the target range					
Preterm infants are often unstable, so it is difficult to keep O_2 saturation in the target range.					
The oxygen isn't adjusted as frequently as needed because the excessive number of alarms, also known as alarm fatigue.					
Pulse oximeters are often unreliable providing the wrong information					
No one has told me the importance of why we need targeted oxygen saturations					

Q13. If you have any other thoughts on targeting oxygen saturations, how we might improve, what are the difficulties, please feel free to add a note below

Appendix 20: Structure of the NeoSat QI educational package

NeoSat QI (Structure of educational package)	
Section	**Contents**
Overview of NeoSat QI project	• Definition of NeoSat QI project • Aim of NeoSat project
Oxygen therapy	• Importance of oxygen therapy • Appropriate oxygen target range
Hazards of Oxygen therapy	• Serious condition associated with SpO2 below target range • Serious conditions associated with SpO2 above target range
Feedback of baseline cycle	• Percentage of time SpO2 kept in target range, below, and above target range • Compliance of setting SpO2 alarm limits correctly

Appendix 21: Clinical observational study parent information sheet.

Intelligent Newborn Monitoring – A feasibility observational study to predict cardiorespiratory changes in neonatal patients

Chief study investigator: Dr Don Sharkey

Parent Information Sheet

DRAFT

Version 2.0

19/3/18

Dear Parent

We are asking if you will agree to take part in a research project to help improve how we monitor newborn babies. It is important to understand why the research is being done and what it will involve for you and your child. So please read this leaflet carefully. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What are we trying to find out with this research?

It can be difficult to identify when a baby's medical condition is changing during the early phases of illness. We use their vital signs, such as heart rate and oxygen levels, along with their clinical assessment to provide us with information on how stable they are. This does rely on the experience of the medical team and understanding when a baby isn't their normal self. The research we want to do is to see if we can teach computer systems to help us identify quicker when babies are becoming unwell using clues from their vital signs and ventilation information. Before we can do this we need to collect the vital signs and clinical care details on 50 babies so we can develop the system.

Why has my baby been invited to participate?

Your baby has been invited to take part because they are going to spend some time on the Neonatal Unit. We are inviting 50 babies to take part.

Does my baby have to take part in the study?

No. It is up to you to decide whether or not to take part. You are free to withdraw from the research at any time without giving a reason. Your decisions about this will not affect the care your baby receives.

What will being in the study involve?

During your baby's hospital stay your clinical care team measure routine vital signs such as oxygen saturations, heart rate and temperature. We will record these details along with details of your obstetric care and your baby's medical care. The research team will record your baby's information during their stay on the neonatal unit. The vital signs will be collected by a computer and analysed at a later date. There will be no changes to the normal clinical care your baby receives as this research is just observing what normally happens.

What will happen to the results of the study?

The study will run for about 2 years. At the end we hope to publish the results so they can be made available to doctors and nurses caring for babies throughout the world. These results will then inform us about the direction we should take further studies to improve care. Your baby will not be identifiable in any publications or presentations. Information from this study may be used for a student thesis for a higher degree. Some of the information we collect could be useful for future research. If you agree, we would also like to safely store this at the University and, where ethical approval is given, use it for such studies. All of the information will be anonymous so you or your baby will not be identifiable.

Who has reviewed the research?

The research has been reviewed by NHS Research Ethics Committee and the Health Research Authority of England for Nottingham University Hospitals NHS Trust.

If you are happy to take part, and are satisfied with the explanations from the research team, you will be asked to sign a consent form. You will be given a copy of the consent form to keep for your records.

What are the possible disadvantages and risks of taking part?

As the research team only collect routine data from the monitors already attached to your baby we do not anticipate any additional risks. All the equipment used to monitor your baby has undergone the same safety tests as other hospital equipment.

What are the possible benefits of taking part?

Your baby will not benefit from being in this study but we hope babies born in the future will benefit from this research.

Will my baby's taking part in the research project be kept confidential?

We will follow ethical and legal practice and all information about your baby will be handled in confidence.

If your baby joins the study the data we collect from your baby will be looked at by authorised people from the University of Nottingham who are organising the research. The data may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to your baby as a research participant and we will do our best to meet this duty.

All information which is collected from your baby during the course of the research will be kept **strictly confidential**, stored in a secure and locked office and on a password protected database. Your baby will be given a unique volunteer number so they cannot be recognised from it. All research data will be kept securely for 7 years. After this time your baby's data will be destroyed as per University protocol.

Who is organising and funding the research?

This research is being organised by the University of Nottingham and is being funded by the Engineering and Physical Sciences Research Council (EPSRC). It will be undertaken at Nottingham University Hospitals NHS Trust. All members of the research team work at the University of Nottingham or Nottingham University Hospitals NHS Trust. No one will benefit financially from this research.

Contacts

- If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions 0115 8230602. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the research team or Hospital Patient Advice and Liaison Service (PALS): Telephone 0800 183 0204.
-

Dr Don Sharkey, Clinical Associate Professor, Tel: (0115) 8230602

Mr Saleh Algarni, PhD Student, Tel: (0115) 8230609

Appendix 22: Clinical observational study consent form.

CONSENT FORM FOR PARENTS
(Version 2.0 19/3/2018)

Title of Study: Intelligent Newborn Monitoring – A feasibility observational study to predict cardiorespiratory changes in neonatal patients

REC ref: 18/EM/0033

Name of Researcher:

Name of Participant (Child): **Please initial box**

1. I confirm that I have read and understand the information sheet version 2.0 dated 19/03/2018 for the above study and have had the opportunity to ask questions. ☐

2. I understand that my child's participation is voluntary and that they are free to withdraw at any time, without giving any reason, and without their medical care or legal rights being affected. I understand that should they withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of my obstetric notes and my baby's medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. ☐

4. I give consent for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my/my baby's participation in this study. ☐

5. I understand that my/my baby's personal details will be kept confidential. ☐

6. Consent for storage and use in possible future research **(<u>Optional</u>)**
   I agree that the data and the information gathered about me/my baby can be stored by the University of Nottingham at the Child Health division, for possible use in future studies. I understand that some of these studies may be carried out by researchers other than the current team who ran the first study. Any data used will be anonymised, and my child will not be identified in anyway. ☐

7. I agree for myself and my child to take part in the above study. ☐

_____	_____	_____
Name of Parent	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____
Name of Principal Investigator	Date	Signature

Appendix 23: Validation of the R script commands identify number of events for each escalation during the 24 hours before respiratory escalation.

Escalation event	Total of desaturations obtained via R script	Total of desaturations checked manually	Total of bradycardias obtained via R script	Total bradycardias checked manually
1	62	62	71	71
2	290	290	67	67
3	32	32	58	58
4	25	25	24	24
5	253	253	148	148
6	262	262	110	110
7	116	116	23	23
8	172	172	17	17
9	62	62	62	62
10	262	262	18	18

Appendix 24: Clinical observational study – details of clinical information of each escalation event.

Escalation Event	24 hours before escalation				48 hours after escalation							
	antibiotic drugs	CRP	Blood Culture	Chest X-ray interpretation	Day 1 antibiotic drugs	Day 2 antibiotic drugs	Day 1 CRP	Day 2 CRP	Day 1 blood culture	Day 2 blood culture	Day 1 chest X-ray	Day 2 Chest X-ray
1	Yes	<5	Negative	Clear	Yes	Yes	<5	N/A	N/A	N/A	Clear	Clear
2	Yes	<5	Negative	N/A	Yes	Yes	<5	N/A	Negative	N/A	Right lung opacification	N/A
3	Yes	N/A	N/A	N/A	Yes	Yes	<5	<5	Negative	N/A	RLL consolidation	N/A
4	Yes	<5	Negative	N/A	Yes	Yes	N/A	N/A	N/A	Negative	RUL and Left lung opacification	Clear
5	Yes	<5	N/A	N/A	Yes	Yes	5	12	Negative	N/A	Clear	N/A
6	Yes	<5	Negative	Clear	Yes	Yes	N/A	N/A	N/A	N/A	Clear	N/A
7	No	<5	Negative	Clear	Yes	Yes	<5	<5	N/A	Negative	Clear	Bilateral peripheral patch airspace
8	No	<5	Negative	Bilateral peripheral patchy air space	Yes	Yes	38	16	N/A	N/A	Bilateral peripheral patch airspace	N/A
9	Yes	N/A	N/A	Grainey bilateral lungs	Yes	Yes	N/A	N/A	N/A	N/A	Clear	N/A
10	No	<5	Negative	N/A	Yes	Yes	<5	<5	Negative	N/A	Lung appear low in volume	N/A