Understanding the adverse impact of centralised care on neonatal outcomes

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Abstract

Centralisation of neonatal intensive care has led to a reduction in mortality, but this has not translated into significant improvements in neurodisability. Understanding the factors contributing to adverse neurological outcomes in infants, especially those transported due to centralised care, could aid the reduction of long-term morbidity in these infants. Centralised care can also risk maternal-infant separation and result in a greater risk of additional stress on the parents of these infants. The aims of my thesis were to: 1) explore the current trends of in-utero transfer (IUT) and early postnatal transportation (PNT) in extremely preterm infants within the UK, 2) evaluate the relationship between early PNT of high-risk infants and severe intraventricular haemorrhage (IVH), 3) quantify the potential stressors for families of extremely preterm infants who undergo centralised care, 4) determine the UK prevalence and treatment of infants with hypoxic-ischaemic encephalopathy (HIE), and 5) explore the effects of whole body vibration (WBV), as experienced during neonatal transportation, on the developing brain.

The prevalence of early PNT has increased over time, which is likely a reflection of a reduction in IUTs and an increase in inter-hospital transfers due to a lack of cot capacity. Early PNT of extremely preterm infants is associated with an increased risk of severe IVH in the first week of life. Centralised intensive care may also have a negative impact on their parents due to the substantial time and distance their baby spends away from their booking hospital. This burden could be further exacerbated as almost half of infants who die are away from their booking hospital and therefore potentially their parents' support network.

HIE is the leading cause of brain injury in the UK and there is an increasing number of infants being managed with therapeutic hypothermia (TH) outside of evidence-based guidance. Almost half of these infants with HIE are born in non-cooling centres without immediate access to TH. Birth in a non-cooling centre is associated with reduced risk of survival without seizures, driven mainly by an increase in seizures.

The transportation pathway exposes both preterm infants and those with HIE to excessive WBV. In a new animal model, short-term WBV exposure results in neuroinflammation, cellular stress and apoptosis in the cortex of the developing brain. The effect of this insult appears to be both more profound in the immature brain and to be dose dependent, potentially indicating a role in the excess brain injury observed in PNT high-risk infants.

In conclusion, centralised neonatal care is associated with a recent increase in early PNT of high-risk infants and adverse neurological outcomes. This could, in part, be a consequence of exposure to excessive WBV during PNT. The additional stress related factors generated by this care pathway could contribute to poor mental health in the parents of these infants. Understanding the factors contributing to these adverse outcomes may help prevent harm and ultimately reduce the hidden burden associated with centralised care on high-risk infants and their families.

Declaration

The work presented in this thesis was carried out at the Academic Division of Child Health, School of Medicine, University of Nottingham between July 2016 and June 2020. I would like to acknowledge Dr Ian Bloor and Dr Hannah Scott (Academic Division of Child Health). Dr Ian Bloor devised the vibration programme used during this project and constructed the custom-made rodent holding chamber. All laboratory training, technical assistance and animal dissection assistance was provided by Dr Ian Bloor. Dr Ian Bloor also carried out S100B analysis for day 21 postnatal samples. Serum corticosterone analysis was carried out by Dr Hannah Scott. All statistical analyses of data within this thesis are my own work. Dr Laila Tata, Dr Lisa Szatkowski and Professor Carol Coupeland have reviewed the statistical methodology used within the attached publications for accuracy and validity.

The dissertation and attached publications are an accurate representation of my own work under the supervision of Clinical Associate Don Sharkey, Dr Jon Dorling (Year 1) and Dr Tracey Farr (From year 2 onwards).

Lara Shipley

June 2020

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Abbreviations

ANS Antenatal steroids

ATF6 Activating transcription factor-6

BH Booking hospital

CHOP C/EBP homologous protein transcription factor

CNS Central Nervous System

CrUSS Cranial ultrasound

CSF Cerebral spinal fluid

DAMP Damage associated molecular patterns

ER Endoplasmic reticulum

GA Gestational age

GFAP Glial fibrillary acidic protein

GRP78 78-kDa glucose-regulated protein

HIE Hypoxic-ischaemic encephalopathy

IL-1ß Interleukin 1 beta

IL-6 Interleukin 6

IL10 Interleukin 10

IL13 Interleukin 13

IRE1 Inositol requiring enzyme 1

IUT In-utero transfer

IVH Intraventricular haemorrhage

ISO International Standards Organization

MCP-1 Monocyte chemoattractant protein-1

NFκB-1 Nuclear Factor Kappa B subunit

NICU Neonatal intensive care unit

NHS National Health Service

PNT Postnatal transportation

RMS Root mean squared

ROI Region of interest

ROS Reactive oxygen species

RT-qPCR Real-time quantitative polymerase chain reaction

TBI Traumatic brain injury

TH Therapeutic hypothermia

TLR4 Toll like receptor 4

TNFα Tumour necrosis factor alpha

TGF-ß Transforming growth factor beta

TUNEL Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling

UPR Unfolded protein response

WBV Whole body vibration

Chapter 1. Introduction

One in seven infants born in the UK require admission to a neonatal unit (1, 2). However, there is growing pressure on these units to deliver essential care due to the increasing trend in the preterm birth rate (3) and survival of infants admitted to neonatal care, especially at the extremes of viability (survival to discharge for infants 22^{+0} to 25^{+6} weeks gestational age (GA) has increased from 40% in 1995 to 66% in 2014) (4). This is most likely a reflection of demographic change in childbearing women (5), advances in antenatal and neonatal care (6, 7), and change in practice towards more proactive management of infants at the lowest GAs (8).

In 2003, neonatal services in England were reorganised into managed clinical networks leading to the development of hospitals of different specialist levels of care, working together with the aim to improve provision of quality care and neonatal outcomes (9). Subsequently, a model of centralised care was established dependant on an infant's GA, clinical condition or requirement for specialised treatment, such as therapeutic hypothermia (TH) for infants with hypoxic-ischaemic encephalopathy (HIE). Centralisation of neonatal intensive care has led to a higher proportion of extremely premature infants being born in units providing the highest level of neonatal care (Level 3 Neonatal Intensive Care Units (NICU)) from 18% (1998-2000) to 49% (2009 to 2010) and a significant decrease in mortality (10). Similar improvements, however, have not been seen in survival without major morbidity, such as intraventricular haemorrhage (IVH), bronchopulmonary dysplasia and necrotising enterocolitis . The EPICure 2 study demonstrated survival without morbidity was not significantly different between extremely preterm infants born in hospitals with level 3 services (11%) compared those with level 2 (8%) (11). Furthermore, there has been a

resultant increase in the total number of postnatal transportation (PNT) both due to centralisation and lack of available cots at higher level neonatal units (10).

In recent years, severe long-term neurodisability has become an increasing public concern due to the substantial impact on both a child's and their family's quality of life. As such, the UK Department of Health has set a target within the NHS Long-term plan to reduce serious brain injury by 50% by 2025 (12). This plan also aims to expand and improve access to specialist mental health care for mother's post-delivery. Several factors have been identified, which could influence the neurological outcomes of infants born outside of NICUs (Figure 1) leading to studies aimed at minimising adverse effects (13, 14). High-risk infants, such as extreme preterms and those with HIE, are managed through a centralised care pathway. These infants already have an increased risk of poor neurodevelopment outcomes secondary to IVH and seizures. It is unclear if there are further adverse effects of this approach both on the infants and their families. This thesis will explore the role of centralised care related factors in relation to outcomes.

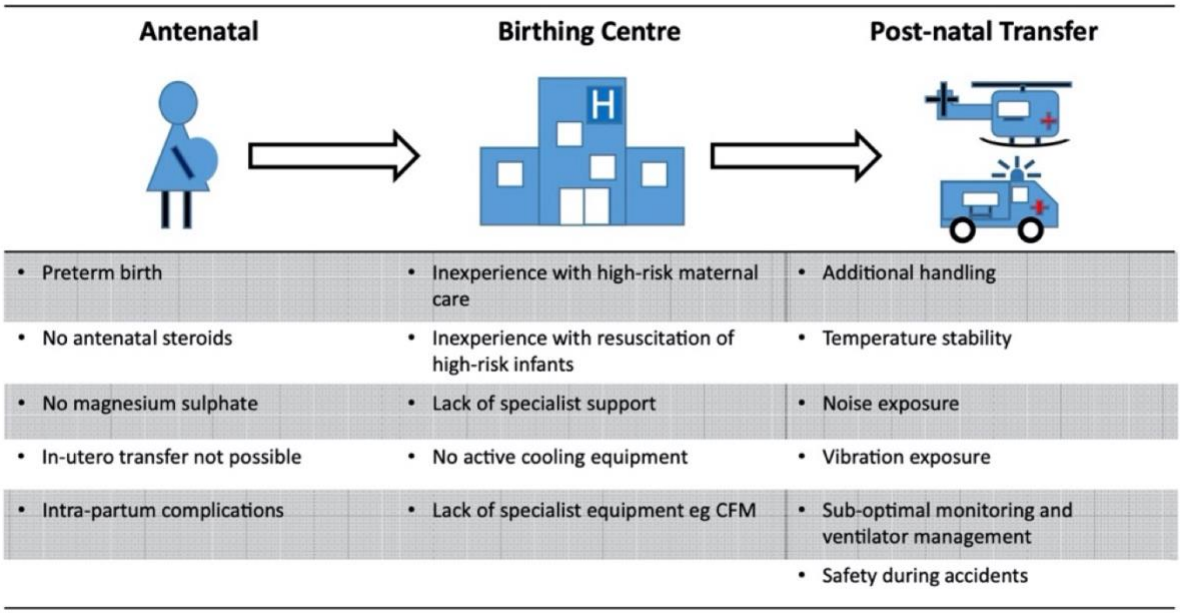


Figure 1: Factors that could affect the neurological outcome for outborn high-risk infants requiring transfer for neonatal intensive care. Taken from Gupta *et al* (13).

1.1. Centralisation of neonatal intensive care for preterm infants

Centralisation of neonatal care advocates transfer of women in threatened preterm labour into higher care centres appropriate for an infants' GA. The EPICure 2 study showed extremely preterm infants who were born in centres with level 3 facilities were 92% more likely to survive without morbidity compared to those who were transferred into the level 3 unit after birth (11). Furthermore, infants who were booked and born at a level 2 centre and did not undergo in-utero transfer (IUT) to a level 3 centre were 44% more likely to die than those who underwent transfer. Since the advent of centralised care, studies have evaluated the association of IUT and PNT with neonatal outcomes in high-risk infants.

1.1.1. In-utero transfers

Several studies have examined morbidity and mortality rates in preterm infants, comparing those who were either born at a centre with a NICU on site (inborn), underwent IUT or had early PNT (11, 15-17). Birth in a level 3 centre and IUT were associated with significantly reduced mortality and severe morbidity (IVH grade 3 or 4 and periventricular leukomalacia) compared to PNT, even following adjustment for key confounding factors (15, 17, 18). However, retrospective studies evaluating IUT compared to inborn or PNT infants have the potential for selection bias due to the characteristics of the women selected for transfer by the obstetrics team. Women with conditions, such as sepsis or antepartum haemorrhage, are less likely to be transferred and ultimately the decision to transfer is dependent on the experience of the obstetrician, rather than set criteria leading to variation. Furthermore, co-ordination of IUT by maternity and neonatal staff consumes a vast amount of clinical time

and often leads to failure due to non-clinical reasons (19). At present, there are no national data on IUTs compared to PNTs in the UK, and if these are changing over time.

Evidence supporting better outcomes in high-risk infants who undergo IUT, compared to PNT, into level 3 centres has resulted in the UK adoption of IUT as the optimal pathway where feasible (20). However, due to maternal clinical instability, imminent delivery and increasing demand on cot capacity, PNT within the first few days of life can be unavoidable.

1.1.2. Postnatal transfers

Approximately 1 in 6 infants <32 weeks GA are transported in the UK within the first 72 hours of life (21), a period coincident with the greatest risk of IVH (22). Previous studies have reported an increased risk of severe IVH with early PNT within the first 72 hours of life, (23-26) but this has not been universal (27, 28). These studies have a number of limitations including omission of important known risk factors for IVH, few high-risk patients and exclusion of neuroprotective agents (ANS and magnesium sulphate). More importantly, these studies were unable to differentiate between early IVH (first 7 days of life) and late IVH (beyond day 7 to discharge from neonatal care), the latter being associated with risk factors potentially unrelated to initial management within the early perinatal period. A better understanding of the association between early IVH and PNT in premature infants could support adoption of better pathways to facilitate IUT and reduce severe morbidity.

1.1.3. Adverse neurological outcomes in preterm infants

Preterm infants have an increased risk of IVH (22, 29). There are several known obstetric, neonatal and environmental risk factors associated with the development of IVH (Table 1). However, the principle cause is due to the fragility of the germinal matrix, a highly vascular

region located next to the lateral ventricles of the brain. Fluctuations in cerebral blood flow can result in pressure changes across the fragile vascular wall leading to haemorrhage (30, 31). Infants <32 weeks' GA are at greatest risk due to the prominence of this fragile vascular region within this age group (30). Administration of antenatal steroids (ANS) are given prior to delivery to reduce respiratory distress syndrome, but they are also associated with a decreased risk of IVH (7). This reduction in IVH could be as a result of better respiratory status (32), which minimises fluctuations in carbon dioxide and resultant vasodilation/vasoconstriction of the cerebral vasculature. However, ANS also upregulate glial fibrillary acidic protein (GFAP), a protein which adds tensile strength to the cerebral vasculature and could potentially minimise vessel rupture (Figure 2) (30). For ANS to be fully beneficial, a full course of ANS should be administered 24 hours to 7 days prior to delivery in women <34 weeks GA with threatened preterm labour (33).

Papile *et al* (34) classified IVH into a grading system based on cranial ultrasound (CrUSS) imaging and post-mortem findings, which correlate well with clinical outcomes. The vast majority of IVHs occur within the first 72 hours of life (22, 35) and have the potential to increase in severity (35). Severe IVH (Grade 3 and 4) has been associated with both short and long-term neurological morbidity and mortality. It has been estimated 50 to 80% of survivors with severe IVH develop cerebral palsy and 70% have cognitive impairment (36). However, low grade mild IVH (Grade 1 and 2) is also associated with lower developmental scores at secondary school age, with a higher percentage of infants requiring educational support. Furthermore, these infants also have an increased risk of moderate/severe neurosensory impairment compared to infants with no IVH (37, 38). This not only can have great impact on the quality of life of both the child and their family, but also on society with the estimated life-time cost for the management of severe neurodisability of £1 million (23).

Additionally, the annual difference in mean public sector cost (health, social and education) is an estimated £2477 greater per child, for extremely preterm infants compared to their term counterparts at 11 years of age (39). Overall, Petrou et al (39) evaluated participants in the UK EPICure study at 11 years of age and found 82.6% of extreme preterm infants had an impairment, compared to 26.2% of their class mates who were born at term (39). Strategies to reduce this level of morbidity are of great importance to public health care and a key driver for government plans.

Obstetric	Neonatal	Environmental
Maternal age	Gender	Transport
Maternal infection	Gestation	Hypothermia
Chorioamnionitis	Low birth weight	
Birth asphyxia	Patent ductus arteriosus	
Prolonged rupture of membranes	Intrauterine growth restriction	
Pre-eclampsia	Mechanical ventilation	
Maternal hypertension	Hyper/Hypocarbia	
Maternal drugs	Acidosis	
Mode of delivery	Sepsis	
Antepartum haemorrhage	Convulsions	
Fetal distress (abnormal CTG/meconium)	Respiratory distress syndrome	
	Pneumothorax	
	Sodium bicarbonate	

CTG, cardiotocography

Table 1. Risk factors associated with the development of intraventricular haemorrhage in preterm infants <34 weeks gestational age. Adapted from Levene *et al* (22).

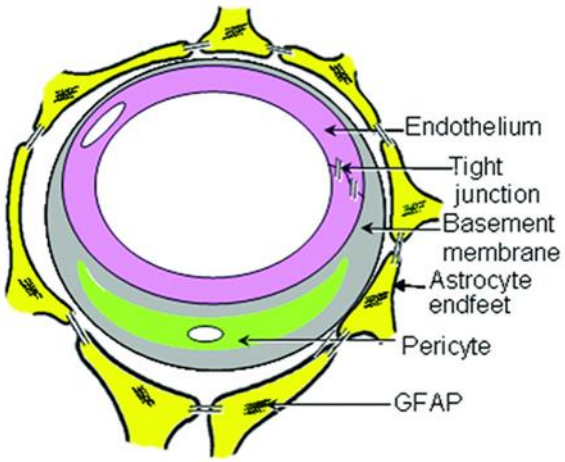


Figure 2. Schematic drawing of the blood brain barrier in cross section showing endothelium, endothelial tight junction, basal lamina, pericyte and astrocyte endfeet. Taken from Ballabh (30).

1.2. Additional burden of centralised intensive care on the family

The birth of a baby is a life event that has a well-established association with postnatal depression and anxiety in parents, with a higher prevalence in the mother (40). Mental health problems such as postnatal depression, not only have a detrimental effect on the parent's quality of life but can also affect their family and may have a long-term effect on their child's behaviour, cognitive and psychosocial development (41-43). Compared to parents of term infants, those of extremely preterm infants have an greater risk of postnatal depression (22.2% vs 41.2%) (40, 44), more family dysfunction and an increased risk of anxiety (45). Currently, 41% (n=38 of 93) of neonatal units surveyed for a recent Bliss report were unable to provide support to parents with a trained mental health worker (46).

The increased psychological stress seen in parents of preterm infants is further compounded beyond the initial postnatal period as preterm infants have a higher rate of brain injury (47) leading to long-term physical, cognitive and neurosensory impairments (48, 49). These co-morbidities impact on family functioning through the need for specialist services, frequent hospital attendances (50), additional educational support (51) and the associated financial burden (39). This is particularly worrying for parents of extremely preterm infants who frequently undergo early PNT, which has been associated with an increased risk of brain injury (52, 53). In addition, transporting an infant from their place of birth to a level 3 centre risks maternal-infant separation. Maternal-infant separation can occur as a result of poor maternal health post-delivery preventing transfer or secondary to physical barriers, such as lack of available transport. This can impact on maternal bonding (54), infant attachment to their caregiver (54), ability to support breast milk expression (55) and lower rates of breast feeding at discharge (55, 56). In the UK, it is unknown how many mothers are separated

from their newborn as a consequence of early PNT. Other pressures, such as the financial implications of regular travel, car parking, accommodation and sustenance, can create further parental stress. In 2014, this was estimated to cost parents £282 per week, or £2256 over their baby's entire hospital stay (57). A recent report found that only 5 of 29 NICUs surveyed had sufficient parent accommodation to meet national guidance (58), resulting in some parents needing alternative residence or frequent trips from their home. A greater understanding of the additional burden associated with the provision of centralised care could help build a case for better funding and allocation of resources, with the aim to reduce mental health problems and deliver better family integrated care.

Although centralisation of care for extremely preterm infants has led to significant reduction in mortality rates since implementation (11), the death of an infant could occur whilst an infant is being cared for away from their booking hospital (BH) leaving parents isolated without their family support nearby, and making bereavement follow up and support more difficult. It is imperative neonatal units are able to provide adequate bereavement support to these parents. There are no recent UK studies exploring the number of preterm infants who die away from their planned delivery hospital. This information is essential to allow development of bereavement services and facilities to accommodate parents during these difficult times.

1.3. Centralisation of neonatal care for infants with hypoxic ischaemic encephalopathy

It is estimated that HIE occurs in about 2-3 infants per 1000 births (47, 59), and is the largest contributor to term neonatal brain injuries in the UK (47). HIE, as described by Sarnat and Sarnat, has been graded as mild (Grade 1), moderate (Grade 2) or severe (Grade 3), based on degree of neurological insult and correlates with prognosis (60). Neonatal seizures are estimated to occur in 50-60% of infants with HIE (61). Infants with moderate/severe HIE, or those who develop seizures within the first few days of life, have a worse prognosis with higher rates of mortality and neurodisability compared to infants with mild HIE or no seizures (60, 62-64). In addition to brain injury attributed to the hypoxic ischaemic insult, there is increasing evidence that seizures can have an additive effect resulting in worse neurodevelopment outcomes compared to those who do not have seizures (65), and this may be independent of HIE severity (64).

TH is an effective and safe treatment for infants $\geq$ 36 weeks' GA with moderate/severe HIE, significantly improving both mortality and survival without major neurodisability at 18 months of age (66-70). The neurological insult associated with HIE is progressive consisting of primary, latent and secondary phases during which excitotoxicity, release of free radicals, cell inflammation and death occur (71). The latent period is considered the optimal time for TH through reduction of glutamate levels, free radicals and reduced apoptosis to prevent secondary programmed cell death and neuro-inflammation (72, 73). However, the length of the latent phase is reduced with severity of HIE, therefore decreasing the optimal window of opportunity for therapeutic treatment (74). Animal studies have demonstrated that TH commenced in the late latent phase results in only partial recovery of cell survival compared

to early use where cell injury and death was significantly reduced (75). These findings are supported by human data reported by Thoresen *et al* (76) who demonstrated TH commenced within 3 hours of age resulted in improved motor outcomes. Furthermore, the TOBY trial highlighted a trend for infants ≥ 36 weeks GA cooled within 4 hours to have a lower risk of death or severe neurodisability at 18 months compared to infants cooled between 4 to 6 hours (66), although the study was not powered to detect this outcome. Following publication of the TOBY trial (66) and subsequent National Institute for Health and Care Excellence guidance in 2010 (77), management with TH is now the standard of care for infants ≥ 36 weeks GA with moderate/severe HIE. In the UK, TH has been implemented at an increasing number of centres (78). However, there is a lack of current UK population data on the prevalence of infants with HIE who are managed with TH. Additionally, there are no up-to-date estimates of the associated mortality rate or proportion of infants who undergo PNT for TH. In order to achieve the ambitious target to reduce severe brain injury, as set out by the UK government within the NHS long-term plan, we need a greater understanding of the at-risk population and factors associated with worse outcomes in these infants.

In the UK, TH management is mainly provided in specialist cooling centres with the experience and capability to manage infants with complex medical needs using specialised automated servo-controlled devices (79). More recently, some level 2 centres are able to commence active TH prior to transportation (80). Infants who are born in non-cooling centres are often passively cooled following a hypoxic insult (66, 81). This is managed through removal of external heating equipment and clothes, cool packs or fans and aims to reduce an infant's core temperature to within optimal therapeutic range. However, this practice can result in over cooling and subsequent increased risk of co-morbidities

(arrhythmias, coagulation abnormalities)(82). Following confirmation of the need for active cooling, transfer to a cooling centre is co-ordinated by designated transport teams. More recently, the proportion of neonatal transport teams who are able to provide active cooling during transportation has increased from 68.4% in 2014 to 87.5% in 2019 (13, 21, 80, 83), leading to an improvement in the number of infants arriving at a cooling centre with a temperature within optimal therapeutic range (83-85). However, the time critical nature for commencing TH and the necessity to transfer infants to cooling centres for on-going management, has increased workload pressure on transport teams (79). Recent changes to practice in the management of extremely preterm infants (8), increasing preterm birth rate (3) and cot capacity issues (86) have all contributed to this increasing demand. Lomax *et al* (80) found the transport team were unable to initiate active TH within the recommended 6 hour window for 20% of infants. Overall, regardless of place of birth, it is well established active TH should commence within 6 hours of birth (76, 77, 87). Surprisingly, there is a paucity of evidence to evaluate whether birth in a non-cooling centre results in delays in achieving optimal therapeutic temperature within 6 hours of birth or impacts on important outcomes.

Centralisation of care for infants with HIE requires those born in non-cooling centres to undergo transfer to a cooling centre soon after their hypoxic insult, potentially at a time of increased susceptibility to secondary brain injury. Transportation of preterm infants in early life is associated with an increased risk of severe brain injury (23, 52, 53). The causation is likely multi-factorial, this in part could be secondary to exposure to adverse conditions, such as excessive vibration (88, 89) during the transport process. It is therefore plausible that the on-going cellular injury seen in infants with HIE (71) could be exacerbated by the

transportation process itself and subsequently affect the outcome. No study has explored the effect of excessive vibration exposure during neonatal transportation on inflammation and cell death pathways within the neonatal brain.

With widespread implementation of TH in the UK (78), there has been increasing concerns that infants are being treated outside of current guidance, for example those with mild HIE or late preterm infants (90-92). Infants with mild HIE have an increased risk of death and adverse neurological outcomes (93) but there is insufficient evidence to establish any significant benefits or harm with the use of TH in these infants (93, 94). Worryingly, studies have suggested that preterm infants with HIE who are treated with TH may have an increased risk of mortality and white matter injury (66, 91). These studies were limited by small numbers, selection bias and lack of preterm comparator group. Treatment of these infants with TH, with the need for transfer to a cooling centre, further exacerbates the burden placed on transportation teams. It may also increase the psychological and financial impact for families during the time an infant is cared for away from their place of birth. There is no current evidence evaluating the magnitude of this problem within the UK population and how this has changed over time. The morbidity associated with the management of infants with HIE outside of current guidance could potentially be reduced with better understanding of the impact of a centralised approach to their care and how this affects outcomes.

1.4. Adverse effects of neonatal transportation

Neonatal transportation is fundamental to the delivery of centralised care. The number of neonatal transfers has increased over time from 10 000 in 2010 to 16 000 in 2016 (86). Furthermore, they often occur at a time when high-risk infants, such as extremely preterm infants and those with HIE, are most vulnerable to potential brain injury (21, 22). Early PNT, within the first 72 hours of life, is associated with a greater risk of severe neurological morbidity in extremely preterm infants (11, 14, 23, 24), as well as potentially in infants with HIE, although there is currently limited evidence in these infants (95). However, the reasons for this are likely multifactorial, such as a result of maternal risk factors, obstetric and early neonatal care, and potentially the transport process itself (96-98). The mechanisms leading to the association of early PNT with worse neurological outcomes are unclear. It has been proposed the adverse environment experienced during the transportation process, such as excessive noise (99-101), whole body vibration (WBV) (88, 89, 102), acceleration and deceleration forces (101) and temperature instability (103) compounds the development of brain injury in these infants.

1.4.1. Whole Body Vibration

Vibration occurs when a motion oscillates around a fixed reference point (equilibrium) and is expressed by frequency, amplitude and acceleration. The number of times the complete motion cycle occurs over one second is referred to as the frequency, which is measured in Hertz (Hz). Vibration can consist of a single frequency or several components of different frequencies occurring simultaneously (e.g. a machine with several parts). The amplitude of a

vibration is a measure of the maximum displacement from zero (equilibrium) and indicates the severity of the vibration (Figure 3).

Throughout the vibration cycle, the velocity of the changing rate of displacement varies from zero (at the extreme peak level) to a maximum (as it passes through its natural equilibrium position) over time (104, 105). Acceleration is a measure of how quickly this velocity changes over time and is expressed in units of metres per second squared (m/s^2). It is the vibration parameter used in human vibration measurements. The root mean squared (rms) acceleration value is the preferred measurement outcome as it encompasses both severity of the vibration and duration over which it occurred. For example, 4 hours of moderate vibration could have more detrimental health effects than 20 min of high exposure. However, if the exposure consists of multiple short and sharp shocks, the rms is less reliable and the peak (maximum amplitude) value should be taken into consideration. Together, the amplitude, frequency and acceleration of vibration can provide the basis for identifying the cause of the vibration and potential detrimental effects of exposure (105).

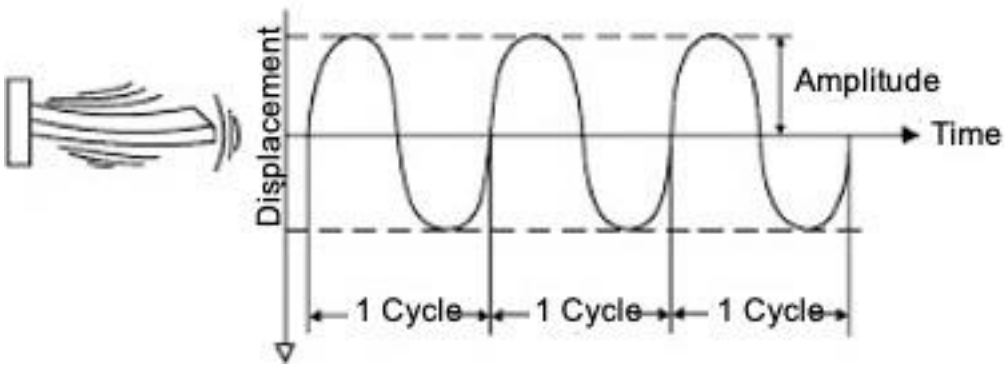


Figure 3. Basics of Vibration. Adapted from Barber (104)

The human body reacts to vibration in a complex, non-linear manner depending on the position of the body and both the direction and frequency of the vibration. WBV is vibration experienced by humans when the body is supported by a vibrating surface. It can affect both comfort and health depending on the magnitude and exposure times. Set standards by the International Standards Organization (ISO 2631) indicate the likely discomfort reactions to vibration levels, expressed as acceleration, for healthy seated adults (Table 2) (106). Levels of vibration greater than 0.315 m/s^2, such as those experienced during operation of heavy goods vehicles, are perceived as uncomfortable and a change in acceleration by 0.1m/s^2 is easily noticeable by the average adult. Unfortunately, there are no standards for neonates or different body positions (e.g. supine, standing). Vibration levels during neonatal transport have been reported in the range of 0.4 – 5.6 m/s^2, which at the upper limit would be deemed extremely uncomfortable for adults (89, 101, 107). Healthcare professionals who accompany neonates during transportation may experience this vibration exposure as motion sickness, fatigue and musculoskeletal problems (108-110).

Acceleration and their perceptions	
Acceleration (m/s^2)	Perception
Less then 0.315	Not uncomfortable
0.315 – 0.63	A little uncomfortable
0.5- 1	Fairly Uncomfortable
0.8-1.6	Uncomfortable
1.25-2.5	Very uncomfortable
Greater than 2.0	Extremely uncomfortable

Table 2. ISO 2631 standards for Whole Body Vibration. Taken from ISO 2631-1 (108)

Vibration can be measured using an accelerometer, which is a sensor that produces an electrical signal proportional to the acceleration of the vibrating component. The WBV measured using an accelerometer is recorded in three directions (X,Y and Z)(Figure 4). The corresponding levels of WBV exposure are expressed as the sum of the vector of these acceleration values (m/s^2 rms).

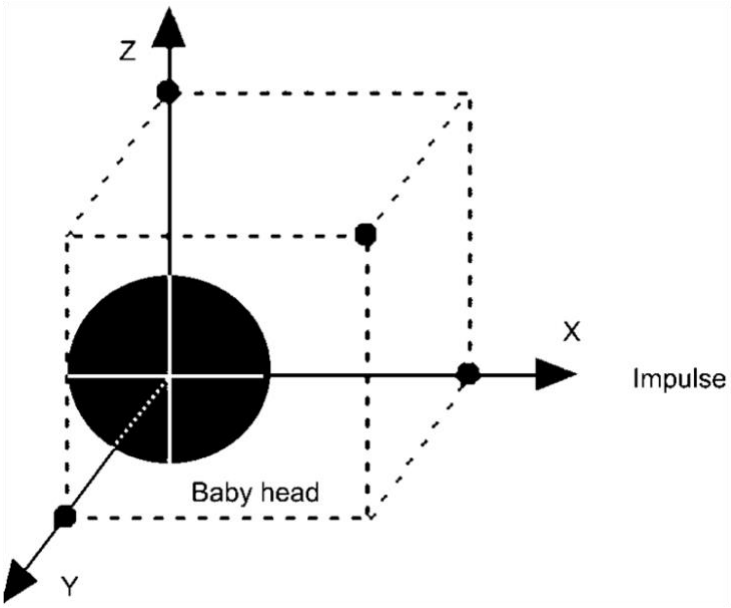


Figure 4. Schematic view of infant in transport incubator with axes defined. Three axes define the movement of the head in space, and the change in acceleration can be divided into constituent vectors along these axes for summation and analysis. Taken from Shah *et al* (111).

Previous studies have evaluated vibration levels during neonatal transport (89, 101, 107) but have been limited by the method of vibration measurement through placement of the accelerometer either on the incubator, the mattress or in a weighted manikin. Placement of an accelerometer on the incubator or mattress may allow estimation of vibration exposure levels during transportation but will not allow measurement of exposure levels experienced by different parts of the body. Additionally, these configurations will not give an indication of how the vibration is transmitted through the body tissues. Likewise, manikins do not exhibit muscle tone, which produce a counter force to vertical vibration. Therefore, vibration is not disseminated through the manikin material in the same manner as body tissues. Blaxter *et al* (88) compared vibration during a small number of UK ambulance transportations (both manikin and neonatal patients (birth gestation range $24^{+2} – 41^{+2}$)) with accelerometers placed on both the incubator and forehead area. They found the mean WBV incubator levels were four times lower than the forehead WBV levels during, hence, underestimating true exposure levels. Vibration in the vertical Z axis (Figure 4) dominated and was on average approximately 0.9 m/s^2. Therefore, this study confirmed previous findings of exposure levels in excess of the 0.5 m/s^2 'action value' threshold for adults in the workplace, as defined by EU regulations (112) during neonatal transportation. Their findings were also consistent with Shenai et al (113), who also used forehead/torso accelerometer placement. Both studies demonstrated that infants transported by ambulance are also exposed to low-frequency vibration levels (3 to 18Hz), which are considered hazardous by adult standards. However, Shenai et al (113) found much higher exposure levels between 2 and 5.6 m/s^2, which could be related to older vehicle age (1980) and American ambulance design. Accurate estimates of WBV levels with concurrent measurement of physiological

parameters during neonatal ambulance transportation, in infants of differing GA and birthweight, will aid further work aimed at minimising detrimental exposure.

1.4.2. Adverse effects associated with whole body vibration

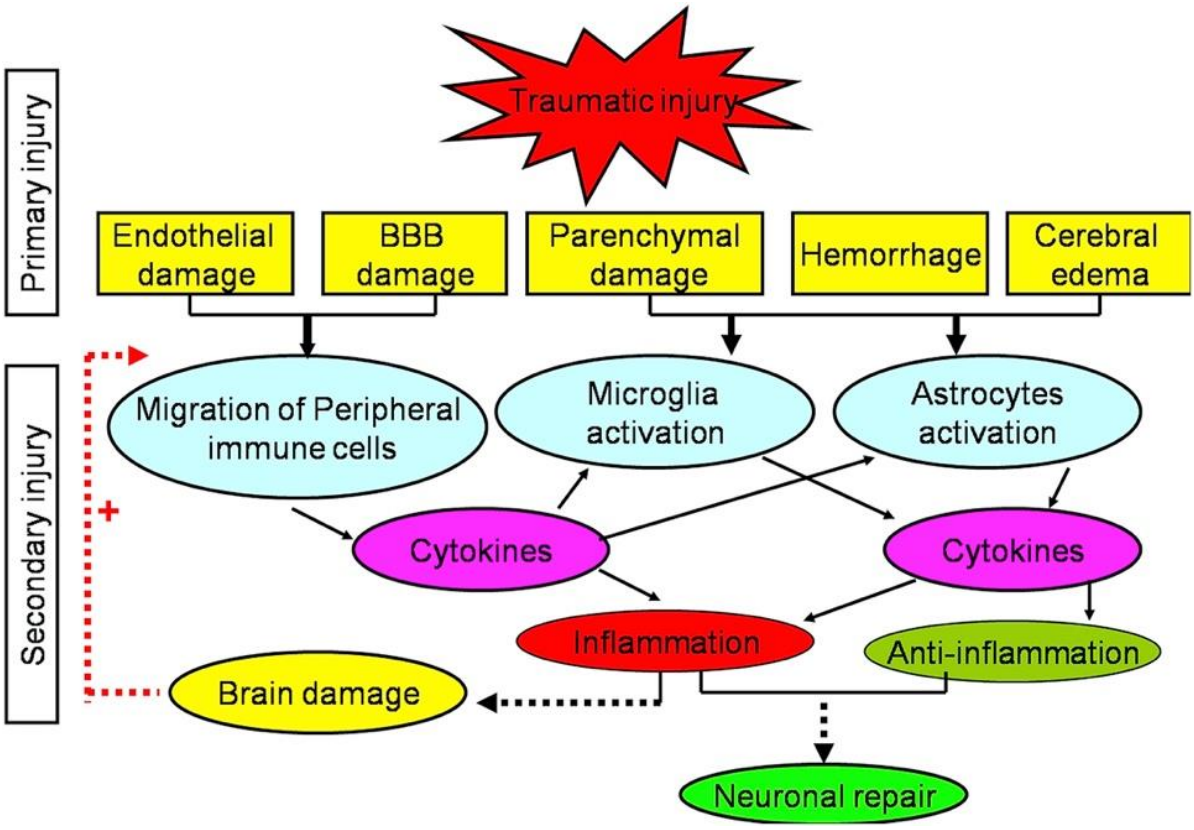
Excessive WBV exposure in healthy adults can cause hypertension, increased heart rate, gastrointestinal disturbance, musculoskeletal problems, reduced alertness, dysregulation of metabolic and endocrine functioning, and neuropathological changes in both central and peripheral nervous systems (109, 110, 114, 115). Variations in cerebral blood flow and uncoupling of cerebral metabolism with WBV exposure has been shown on positive emission tomography (PET scan) in healthy adult volunteers (116, 117). It is plausible that the excessive WBV experienced during neonatal transportation could also lead to changes in cerebral blood flow resulting in injury to the fragile vessels within the germinal matrix and increase the risk of IVH in these high-risk infants. An area of additional interest is the role of excessive WBV exposure resulting directly in brain tissue injury and subsequent neurological impairment. This theory is supported by animal studies. Yan *et al* (118) used an adult rodent model to explore the effect of chronic WBV on cerebral vasculature, cognition and physiology. The effects of WBV were found to be insidious and resulted in reproducible sequelae when assessed over time. Acute, short-term exposure (4.9 m/s^2 for 4 hr/day, 5 days/week, for a total of 2 weeks) caused cerebral vascular vasospasm followed by constriction with increased free radicals. Further prolonged exposure resulted in thickened cerebral vascular walls, which became uneven with loss of endothelial cells and internal elastic membrane lining the vessels and glial cell oedema (118). Additionally, chronic exposure resulted in deterioration in the behaviour and physiology of the rats who showed a cognitive decline (inability to complete a maze compared to pre exposure), reduced grip

force (indicating injury to motor fibres) and extended flick test (time taken to withdraw the tail from a heat stimulus indicating sensory nerve damage). Moreover, Curry *et al* (119) found endothelial thinning and loss after a single 4-hour exposure to WBV and vasoconstriction within 5 minutes, suggesting vascular injury is an early event. The exact mechanism of injury secondary to WBV is uncertain, though potentially transmitted vibrational energy within the tissues could result in shearing or twisting forces, triggering the inflammatory and cell death pathway (118). Additional injury could occur during neonatal transportation secondary to impaction of the brain against the cranium if an infant's head is not adequately secured. Although these studies have focused on exposure to chronic WBV, they still provide valuable evidence that WBV can result in brain tissue injury. Zeeman *et al* (120) found exposure to short periods of WBV resulted in both activation of microglia and upregulation of GFAP, a biomarker of neuronal injury (121), in the spinal cord of rats and resulted in sustained limb allodynia (defined as increased limb sensitivity to stimulation through to study end point on day 14 post exposure). Exploring the role of short-term WBV exposure in relation to potential brain injury and identification of genes implicated in development of pathophysiology could be key to understanding part of the mechanism for the increased risk of brain injury associated with transported high-risk infants.

1.5. Role of inflammation and microglia in brain injury

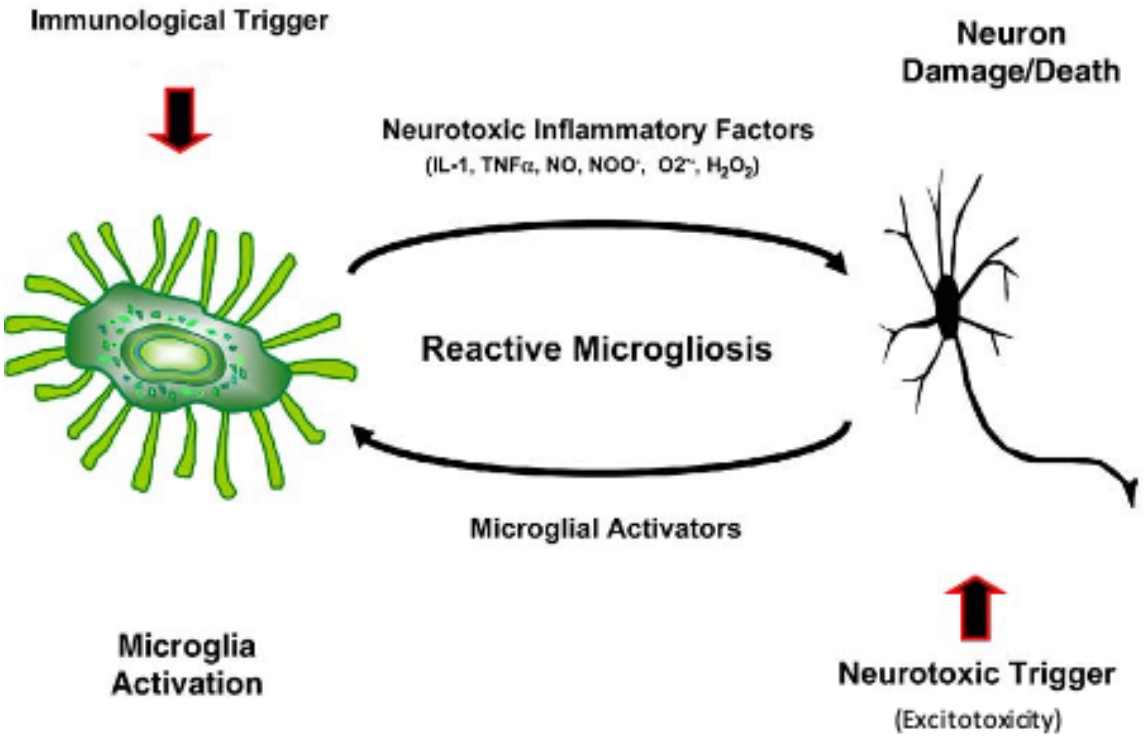
Brain injury resulting in neuropathology has historically been described as occurring in two stages. Primary tissue damage, which occurs as a direct result of an insult such as trauma or hypoxia, followed by delayed secondary tissue damage due to the brain's inflammatory response, development of oedema and subsequent cell necrosis or apoptosis (122). The release of inflammatory mediators following primary injury can contribute to neuronal death and activation of microglia (123, 124) (Figure 5). Microglia are tissue-based macrophages and key innate immune cells of the central nervous system. They have an important role in programmed neural cell death during early postnatal brain development (125) and in regulating developmental synaptogenesis (126). Following injury, initial phagocytosis of cell debris at the site of injury by microglia is beneficial. However, during the subsequent phases of injury microglia release pro-inflammatory and cytotoxic factors (Interleukin-1 beta (IL-1ß), Interleukin 6 (IL-6), Tumour Necrosis Factor alpha (TNFα), Monocyte chemoattractant protein-1 (MCP-1) and Reactive Oxygen Species (ROS))(127). These inflammatory mediators, as well as damaging the white matter within the brain (128), further activate additional microglia and other inflammation promoting cells. This causes migration of more microglia to the site of injury and initiates a self-perpetuating cycle of neuronal death (Figure 6)(127). Although, the inflammatory response in excess can contribute to brain injury, it is necessary for regenerative and reparative processes (122). Activation of the microglia following primary insult can result in two different phenotypes, a M1 pro-inflammatory phenotype or a M2 anti-inflammatory phenotype (129). The M2 phenotype results in the release of anti-inflammatory factors (Interleukin 10 (IL-10), Interleukin 13 (IL-13) and Transforming growth factor beta (TGF-ß)) (127). The ability to

change between these phenotypes is essential for initiation of cellular repair and damage control. Toll-like receptors, which are present on the membrane of microglia respond to endogenous factors (damage associated molecular patterns, DAMPs) released by damaged cells and play an important role in the regulation of M1 and M2 microglia (129). It has been suggested that activation of Toll-like receptor 4 (TRL4) leads to upregulation of the pro-inflammatory M1 phenotype, as gene knockout results in an increased M2 to M1 phenotype ratio (130). Furthermore, the role microglia perform following injury depends on the type of insult and progression of the injury within the tissue (126, 127). Neuronal cell death is essential for removal of dysfunctional cells post-insult, however, excessive cell loss can lead to neurological deficits (131). It has been suggested milder insults may allow cells to recover, otherwise damaged cells will undergo apoptosis potentially several days post injury (132, 133). Despite evidence associating both brain tissue injury following an insult with the activation of the inflammatory and cell death pathways (122), and singular short-term WBV exposure with the activation of microglia within the peripheral nervous system(120), there have been no studies exploring the potential relationship between these processes.



BBB, Blood brain barrier

Figure 5. Schematic diagram of primary and secondary brain damage after traumatic brain injury, and the involvement of peripheral immune cells, microglia, astrocytes and endothelial cells. Taken from Shi *et al* (129).

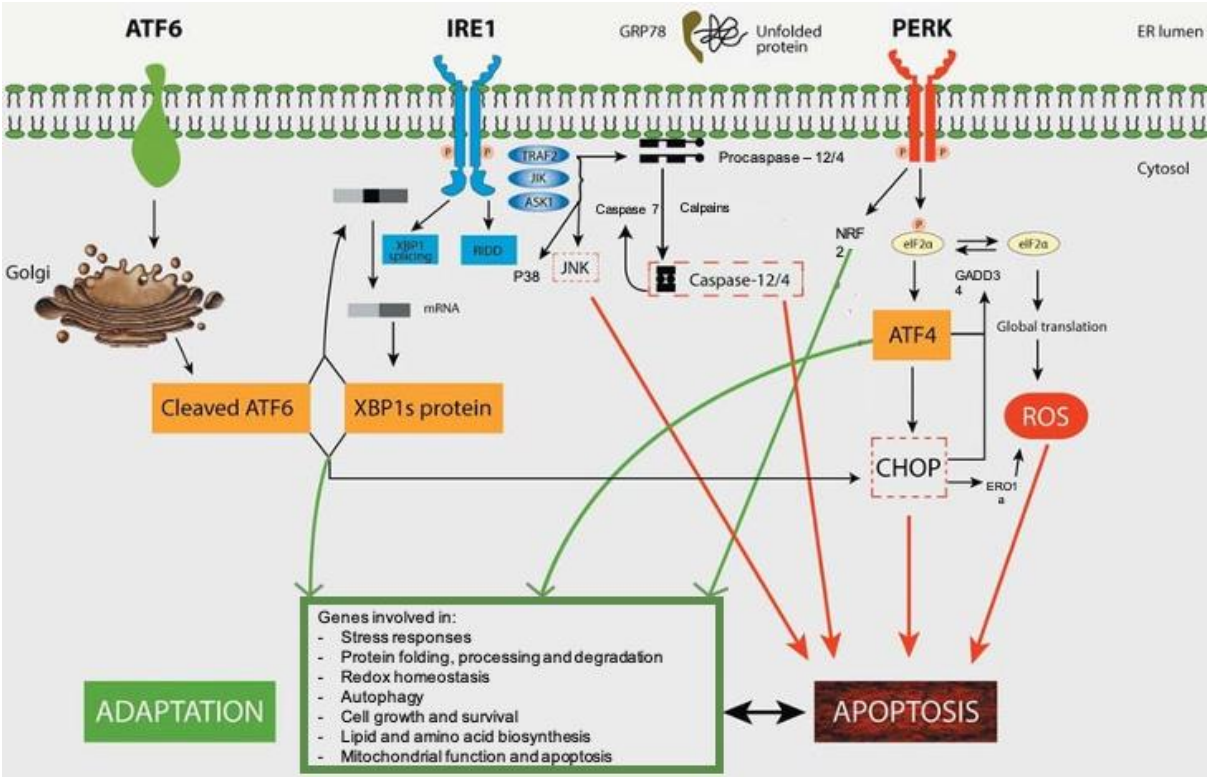


IL-1, Interleukin 1; TNFα, Tumour Necrosis Factor alpha; NO, Nitric oxide; NOO^-, Peroxynitrite; $O2^-$, Superoxide; H_2O_2, Hydrogen peroxide

Figure 6. Reactive microgliosis is a self-propelling cycle of neuronal damage. Regardless of the initial toxic insult, dying or damaged neurons activate microglia to produce neurotoxic factors, which are toxic to surrounding neurons. Adapted from Block et al (127).

1.6. Role of endoplasmic reticulum stress and cell death pathways in brain injury

Cell death following traumatic brain injury (TBI) has been associated with both long-term neurological deficits and mortality in both adults and children (131, 134, 135). The mechanisms leading to activation of cell death pathways following inflammation are complex and likely to be multifactorial. The activation of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) post TBI, with subsequent cell apoptosis, is associated with poor long-term neurological outcomes, making it a potential area to develop targeted preventative therapies (136). The ER is a cellular organelle involved in the folding and assembly of proteins and membrane lipids (137). Following TBI, both direct injury to cells and activation of inflammatory pathways lead to an increase in ROS and intracellular calcium within the cell cytoplasm (136). These events cause proteins to unfold, which eventually overwhelms the ability of the ER to re-fold. The unfolded proteins accumulate within the ER lumen, driving ER stress and the UPR. The UPR is a collective term used to describe three mechanistically distinct intracellular signalling transduction pathways. ER stress and UPR aim to maintain homeostasis in the ER and ultimately cell survival through 1) inhibition of protein synthesis to slow down protein production giving the ER time to refold unfolded proteins 2) manipulating stress response genes involved in protein refolding 3) degrading beyond repair unfolded proteins (137). However, persistent UPR activity indicates ER stress cannot be controlled and results in cell apoptosis (Figure 7) (138).



ASK1: apoptosis signal-regulating kinase; CHOP: C/EBP homologous protein transcription factor; ERAD: ER-associated protein degradation; ERO1α: ER oxidase 1α; JIK: jun kinase-inhibitory kinase; ROS: reactive oxygen species; TRAF2: tumour necrosis factor receptor-associated factor-2.

Figure 7. Summary diagram demonstrating activation of the unfolded protein response (UPR) following endoreticulum (ER) stress. ER stress induces the UPR through a triple transcription factor system. Misfolded proteins sequestrate 78-kDa glucose-regulated protein (GRP78), thus allowing the activation of three ER membrane-associated proteins. Activating transcription factor-6 (ATF6) translocates to the Golgi for cleavage, subsequently regulating UPR gene expression. Inositol requiring enzyme 1 (IRE1) upregulates genes associated with protein degradation, decreases mRNA translation and ultimately activates proinflammatory and apoptotic pathways. Protein kinase RNA like endoplasmic reticulum kinase (PERK) phosphorylates eukaryotic initiation factor 2α (eIF2α) to attenuate global translation. C/EBP homologous protein (CHOP) signals apoptosis following prolonged activation. Adapted from Vandewynckel *et al* (139).

Alternative cell death pathways implicated in injury-induced neuronal loss secondary to apoptosis are caspase-dependent pathways and activation of the tumour suppression gene, p53, following DNA damage (140). Caspases are a group of proteases and play an essential role in the apoptotic pathway (134). Caspase 3 has been shown to be the main "executioner" caspase implemented in neuronal apoptosis post-TBI (134, 141, 142). Interestingly, treatment of rodents with caspase inhibitors improves neurological deficits post-traumatic insult (141). Furthermore, DNA fragmentation secondary to direct cellular damage, ROS and activation of downstream caspase-3 dependent protein (143) can lead to induction and upregulation of p53 gene. P53 acts to either promote cell survival through repair of damaged DNA or can initiate cell apoptosis (140).

Overall, studies evaluating the ER stress response, cell death pathways and subsequent neurological outcomes secondary to TBI have mainly focused on animal models of injury such as fluid percussion injury, controlled cortical impact, weight drop impact and concussion models (144). These models are likely to produce different pathophysiological responses to traumatic insult than animals exposed to WBV. Exposure to chronic WBV has been associated with neuronal apoptosis and subsequent adverse neurodevelopmental outcomes (118); however, the mechanisms linking WBV to cell damage are unclear. No studies have explored the effect of a single, short episode of WBV exposure on the activation of cell death pathways in the brain, which is essential to understand long-term outcomes and potential strategies for therapeutic intervention.

In summary, it is neither ethical to expose high-risk infants to excessive WBV exposure to evaluate the effect on neurological outcomes nor possible to directly measure markers of

neuroinflammation or cell death easily in infants who routinely undergo inter-hospital transfers. Therefore, an appropriate animal model representative of both preterm and term infants is required to evaluate these potential associations.

1.7. Development of a rodent model to evaluate vibration related brain injury

Rodents have been used as models for research to represent both normal and pathological processes of the human brain for centuries. Anatomically, the structures of the human and rodent brain are similar and from a practical view rats are easy to handle, relatively inexpensive and less prone to stress through handling than other species (145) (Figure 8).

There is currently debate as to the developmental stage of the rodent brain which best correlates with those of human infants. Historically, day 7 (D7) rodents have been used in research to represent the central nervous system (CNS) of term infants following comparison of the timing of the brain's peak growth spurt between rodents and term infants (146). However, this has been challenged due to the varying development rate in different areas of the brain (147). Additionally, the use of anatomical weight alone does not take into consideration functional maturation of the brain (147). Romijn *et al* (148) and Clancy *et al* (147) evaluated neurological events to determine the best-fit model to correlate the postnatal age of rodents with a human infant. They concluded rodents of postnatal day 12-13 were functionally the best-fit comparison to a term human infant.

More recently, Craig *et al* (149) proposed an alternative view by encompassing the outcome area of interest into the model. They proposed a rodent model based on the comparison of cerebral white matter development in rodents and preterm infants. They concluded postnatal D7 and D14 rodents are comparative to human infants of 32 weeks' GA and at term, respectively, for CNS development (Figure 9). This model has been used in the research of neonatal brain injuries (e.g. IVH and HIE) (149). Furthermore, Rice *et al* (150) supported these conclusions by demonstrating a D7 rat pup has a germinal matrix and

cortical layering similar to a 34 week human infant, therefore, allowing this age to be used in studies of germinal matrix haemorrhage.

Overall, brain development can be classified by anatomy, velocity growth curve, neurophysiological, metabolic and electrical activity. For work related to my thesis, I have used rats at postnatal D4, 7 and 21 to represent human neonatal brains at 24-25 weeks, 32-34 weeks and post-term gestations, respectively. I have based this on the anatomy of the germinal matrix, neurological function and development of white matter, which are my key areas of interest.

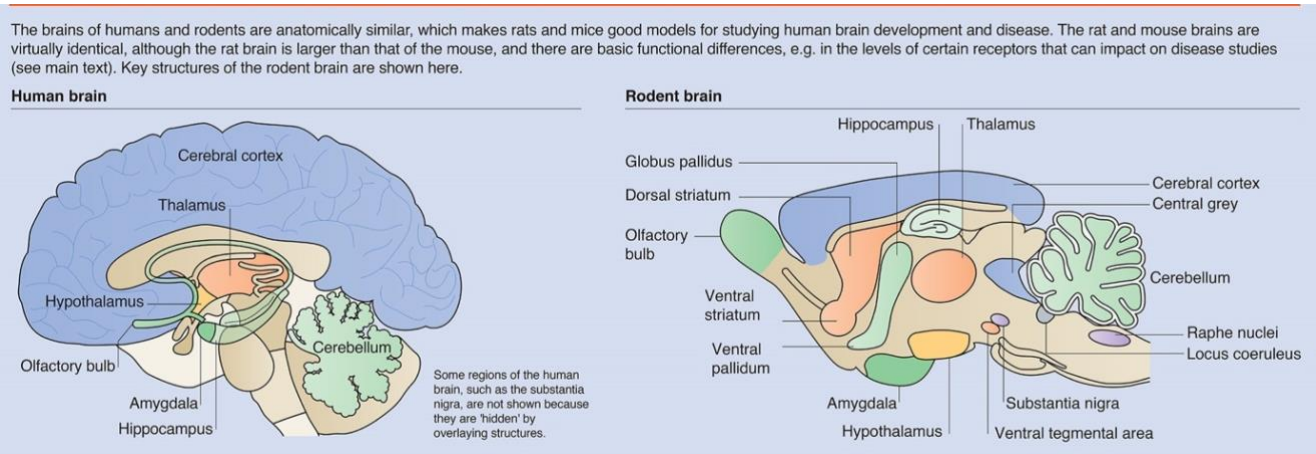
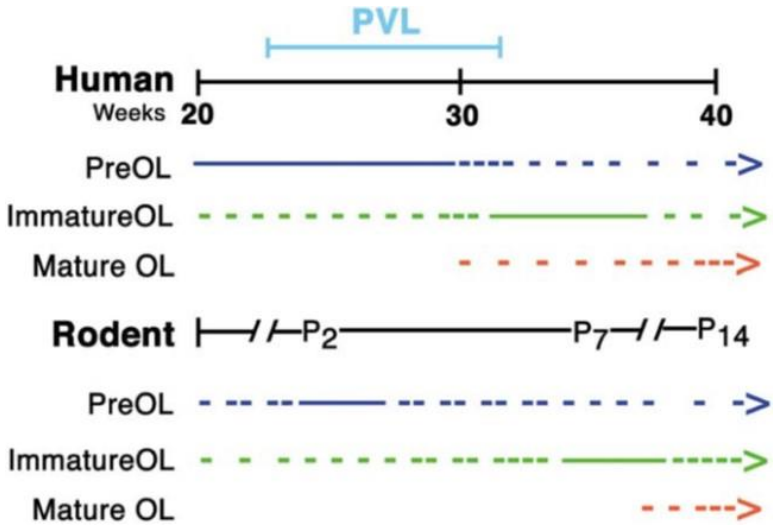


Figure 8: Comparison of human and rodent brain anatomy. Taken from Ellenbrook *et al* (145).



PVL, periventricular leukomalacia; OL, Oligodendrocyte; P2, postnatal day 2; P7, postnatal day 7; P14, postnatal day 14

Figure 9. Summary diagram comparing the salient features of human versus rodent oligodendrocyte (OL) lineage progression. Human is depicted during the latter half of gestation (20–40 weeks) and is based upon data from Back *et al* (151). The windows in fetal human white matter development that correspond with the perinatal rodent at postnatal day P2 and P7 are depicted. Solid lines indicate the developmental period when a given OL stage predominates and dotted lines indicate the period when that stage is a minor constituent. Taken from Craig *et al* (149).

1.8. Biomarkers of neurological injury

Animal models are essential to gain an appreciation of the potential causative mechanisms underlying brain injury in response to specific adverse insults and aid development of preventative therapeutic interventions. However, there are several limitations with the use of animal models to represent human neuropathophysiology and correlation with translation into potential human outcomes. More recent studies have showed promising results using biomarkers in human subjects, including infants, to detect brain injury following either hypoxic or traumatic insult. These can be easily detected in the serum and urine and have been shown to correlate with prognosis (121, 152-154). Furthermore, biomarkers of brain injury have been shown to be effective in detection of sub-clinical injury at stages when imaging or monitoring procedures have remained negative (121, 155).

1.8.1. S100B

S100B is a calcium binding protein, which is concentrated in glial cells, astrocytes, neurons and Schwann cells. It is released during brain cellular injury (156, 157) and can be detected in CSF, blood (via the blood brain barrier), urine and saliva (158). S100B is associated with increased levels in infants with IVH, even prior to ultrasound detection, and positively correlates with the severity of IVH (154, 155).

1.8.2. GFAP

GFAP is a cytoskeleton protein expressed by astrocytes and ependymal cells to help maintain mechanical strength of the blood-brain barrier. Injury to neurons as a result of inflammation or trauma results in a reactive increase in the number of astrocytes with

subsequent up regulation of GFAP protein. In addition, GFAP is released rapidly out of damaged astrocytes and ependymal cells and can subsequently be measured in both the CSF and blood (121).

Both S100B and GFAP could be used as biomarkers of potential subclinical brain injury as a result of exposure to WBV during ambulance transfer and potentially identify those who are at increased risk of adverse outcomes even when imaging is normal.

1.9. Hypotheses and aims

The overarching hypotheses relating to this thesis are:

- Centralisation of neonatal care is associated with adverse neonatal outcomes in high-risk infants, which in part is secondary to exposure to noxious stimuli, such as WBV, during the transportation process.
- Centralisation of neonatal care can create an additional burden on parents which could increase the risk of poor mental health.

The aims of my thesis were:

- Establish the UK prevalence of extremely preterm infants who undergo either IUT or early PNT
- Explore the risk of IVH in extremely preterm infants who undergo early PNT
- Investigate potential stressors for families of high-risk infants who undergo centralised care
- Determine the prevalence of the at risk population of infants with HIE within the UK
- Determine the effects of centralised care on short-term neurological outcomes infants with HIE
- Explore the effects of short-term WBV on the developing brain at cellular level, using a novel rodent model

The submitted and drafted papers contained within this thesis, as well as the following chapters contain in-depth detail of experimental groups, methods and statistical analysis for each presented study. The studies presented within this thesis are:

1. **Paper 1. Temporal trends of in-utero and early postnatal transfer of babies born 23^{+0} to 27^{+6} weeks' gestation between 2011-2016: A UK population study.**

   Retrospective epidemiological study of preterm infants 23^{+0} to 27^{+6} weeks' GA to define the prevalence of infants who underwent either IUT or PNT within 72 hours of birth in the UK and any temporal changes.

2. **Paper 2: Risk of severe intraventricular haemorrhage in the first week of life in preterm infants transported before 72 hours of age**

   Retrospective epidemiological study of preterm infants <32 weeks' GA, who were either born and cared for at a level 3 centre or required transfer between centres within the first 72 hours of life. Multivariable logistic regression models adjusting for key confounders were used to determine the risk of IVH in transported infants (52).

3. **Paper 3. Quantifying the impact of centralised neonatal care on the family: A national population study.**

   Retrospective observational cohort study of all infants <28 weeks' GA admitted to UK

neonatal units. Quantification of potential stressful factors, unique to centralised neonatal care, on the family of high-risk infants.

4. **Paper 4. Trends in the prevalence and management of hypoxic-ischaemic encephalopathy in the therapeutic hypothermia era: a national population study**

   Retrospective observational cohort study of infants admitted to neonatal units in the UK to define the prevalence of the at-risk population of infants with HIE and changes over time.

5. **Association of birth in centres without active therapeutic hypothermia and seizure-free survival following neonatal hypoxic ischaemic encephalopathy: A nationwide study**

   Retrospective observational cohort study of infants ≥36 weeks GA with moderate/severe HIE and underwent TH. Propensity score-matching was used to determine the association of birth in a non-cooling centre with survival without seizures, mortality and seizures alone.

6. **Pre-manuscript data: The effects of WBV, as experienced during neonatal ambulance transportation, on the developing brain**

   Animal study using a novel neonatal rodent model that I helped develop to investigate the potential adverse effects of WBV exposure at a level equivalent to that experienced

by a neonate during an ambulance transfer. The effect of WBV on cortical injury within the brain at different developmental stages was evaluated.

Chapter 2. Temporal trends of in-utero and early postnatal transfer of infants born 23^{+0} to 27^{+6} weeks' gestation between 2011-2016: A UK population study

Following the implementation of centralised intensive care in the UK, national guidance was developed advising high-risk extremely preterm infants (<28 weeks' GA) should be delivered and cared for in hospitals with a level 3 NICU on site due their inherent increased risk of mortality and severe morbidity (159, 160). Additionally, neonatal services should aim to configure services to achieve this goal in 85% of extremely preterm deliveries (1). IUT is the optimal pathway, where feasible, based on evidence supporting better outcomes compared to PNT as previously discussed (15-17, 20). However, there is no current national data evaluating the rate of IUT or PNT in the UK or whether this has changed over time. Furthermore, there is a lack of national data to evaluate trends in early PNT between hospitals of similar levels, which could provide valuable information on cot capacity issues. Therefore, I have evaluated the prevalence of IUT and early PNT (within 72 hours of birth) including the level of referring and receiving neonatal units within the UK and changes over time. This will allow a greater appreciation of current practice in the UK and potential impact of lack of cot capacity on PNT rate.

For statistical analysis, the study population was separated into two equal epochs to evaluate temporal changes. Mann-Whitney U test was used for group analysis to compare changes between epochs. Odds ratio (OR) and confidence intervals (CI) were calculated.

During the study period from 2011 to 2016, there were 14 719 infants 23^{+0} to 27^{+6} weeks' GA admitted to neonatal units in the UK. Of these, 4005 (27.2%) underwent IUT and 3042 (20.7%) had early PNT. The prevalence of IUT has significantly decreased over time (between Epoch 1 (2011 to 2013) and Epoch 2 (2014 to 2016)) (28.3% (n= 2089) vs 26% (n=1916), p<0.01), whilst conversely early PNTs within 72 hours have increased (19.8% (n=1461) vs 21.5% (n=1581), p=0.01). The total percentage of all IUT and PNTs by GA between the two epochs are shown in Figure 10 and Paper 1. Gale *et al* (19) examined potential barriers to IUT locally within the London area and found the main reasons were a considerable length of time required by labour ward staff to organise, lack of a co-ordinated system to find a neonatal cot and maternal bed within the same hospital, and concerns regarding imminent delivery. Careful consideration is required to determine which women can be safely transferred using obstetric expertise and predictors of preterm delivery (161, 162). However, evidence suggests the majority of women in threatened preterm labour do not deliver within 24 hours of presentation (163, 164). Additionally, infants rarely deliver during transportation (161, 163, 165). This highlights the window of opportunity for IUT may be greater than currently perceived.

On the other hand, some women who undergo IUT for threatened preterm labour do not subsequently go onto deliver within 7 days, with many delivering at gestations which would no longer require level 3 care (166). These transferred women could contribute to maternity unit bed pressures and neonatal cot capacity issues due to expected deliveries potentially being considered in bed numbers. In addition, studies have demonstrated IUT can have a negative psychological impact on families due to loss of social network support, separation from other children, financial costs associated with relocating to another centre and loss of choice (167-169). Future work evaluating the additional stress and burden placed on these

families would help drive quality improvement projects to provide support for these families and develop shared decision making pathways.

Previous studies have sought to establish risk factor scoring tools to predict the probability of preterm birth, though few have been used in relation to IUT (162, 170). An on-going study (EQUIPTT) may provide much needed evidence for effectiveness of the use of these models as clinical decision-making aids to balance the risk of preterm birth versus inappropriate transfer of women (171). However, in this study I was unable to ascertain the number of mother's who had IUT but did not subsequently deliver and infants who were IUT but died in the delivery room, as these data are not recorded within the NNRD. For this reason, it is essential the national trends of women who undergo IUT but do not imminently deliver are evaluated to inform future guidance on who and when to transfer.

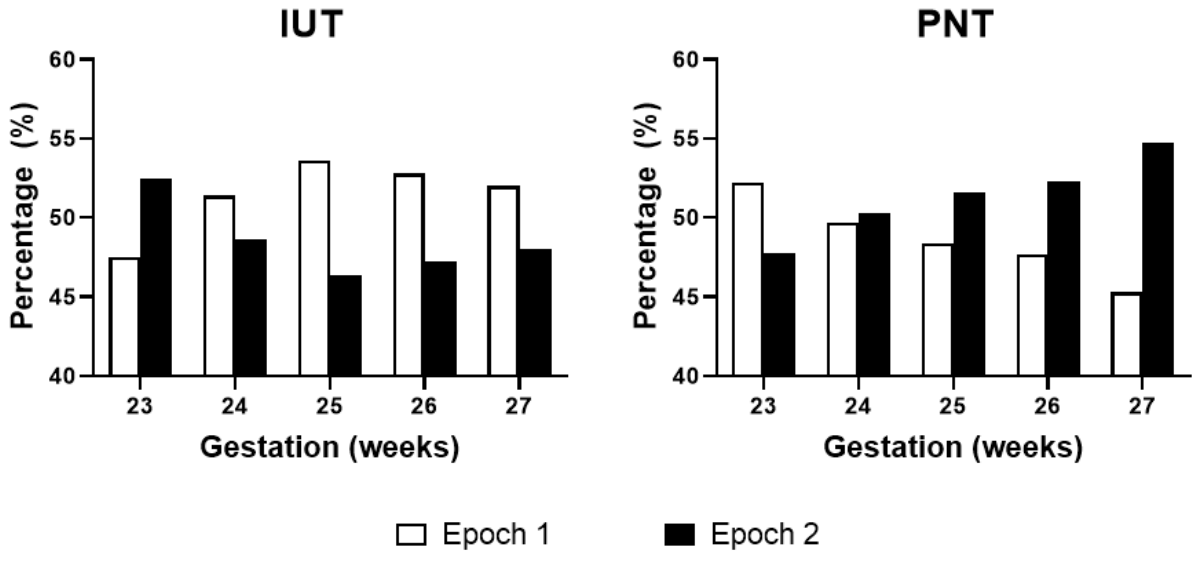


Figure 10. Comparison of in-utero (IUT) and postnatal transfers (PNT) within 72 hours of life between Epoch 1 (2011-2013) and Epoch 2 (2014-2016) by GA (IUT n=4005, PNT n=3042). Taken from Paper 1

My findings of a significant increase in PNTs could expose a greater number of vulnerable infants to an increased risk of severe IVH (14, 23, 52). The increasing trend in PNT maybe as a consequence of difficulty in undertaking IUT and lack of cot capacity (46). Additionally, staff shortages (46), increasing preterm birth rate (3), as well as improved neonatal intensive care and changes in practice (172, 173) meaning more infants are surviving, are likely to contribute to cot pressures and therefore the need for PNT.

The proportion of infants who undergo early level 3 to level 3 PNTs has increased over time (8.1% (n=119) vs 10.2% (n=161), p=0.05)(Table 3) and Paper 1. Although the exact reason for transfer is beyond the scope of this study, it potentially could reflect transfers due to lack of cot capacity and highlights the impact of cot capacity issues on the expanding PNT rate. Furthermore, the overall rate of mortality for extremely preterm infants has significantly decreased over time, however, for infants who underwent level 3 to level 3 early PNT there was a significant increase in mortality. Given these infants should receive similar standards of care at both centres and there was no difference in demographic background or ANS administration between time epoch groups, this raises concern regarding early PNT as a contributing factor in this high-risk group. However, this study was limited by the use of retrospective methodology, so I was unable to account for evolution of practice over the study period and the lack of detail on the cause of death in these infants. Further work is warranted to further validate these findings and to explore whether this could be, in part, as a result of exposure to excessive WBV during PNT. In addition, my findings provide support that funding and strategic service change could help address factors contributing to cot capacity issues. Development of national guidance to aid clinical

decision making about which infant to transfer due to capacity related issues may minimise potential risks.

The lack of detailed clinical background information for infants who had early PNT restricted my ability to assess whether there were missed opportunities for IUT. Despite this, I was able to evaluate trends in proportion of PNT infants who received a full course ANS. This would suggest the mothers of these infants were an inpatient at the referring centre for at least a period of 24 hours prior to delivery, during which an IUT may have been feasible. My findings showed the proportion of early PNT infants who received a full course of ANS has significantly increased over time (40% (n=578) to 43.3% (n=685), p=0.02). This data supports findings from Gale *et al* (19) that a large number of IUT attempts fail due to service provision barriers.

Given the significant reduction of IUTs resulting in preterm delivery over time, a review of national service configuration is required and consideration be given to a designated centralised approach to IUT to avoid unnecessary early PNT. My findings highlight the potential need for formal guidance upon which IUT decisions are made for women in threatened preterm labour to minimise missed opportunities and un-necessary transfer of women. Furthermore, it is important neonatal networks develop strategies to evaluate service provision and address barriers to IUT. This will help to achieve government targets and improve outcomes.

Unit level		Epoch 1 n = 1461	Epoch 2 n = 1581		
Referring	**Receiving**	**PNT infants n (%)**	**PNT infants n (%)**	**% change**	**p value**
L3	L3	119 (8.1)	161 (10.2)	+ 35.3	0.05
L2	L3	902 (61.7)	1022 (64.6)	+13.3	0.09
L1	L3	317 (21.7)	279 (17.6)	-12.0	<0.01
L3	L2	25 (1.7)	31 (2.0)	+24.0	0.61
L2	L2	36 (2.5)	32 (2.0)	-11.1	0.41
L1	L2	34 (2.3)	22 (1.4)	-35.3	0.06
MLU	L2	2 (0.1)	0 (0)	n too small	-
MLU	L3	16 (1.1)	14 (0.9)	n too small	-
Missing	Missing	9 (0.6)	19 (1.2)	-	-

L3, Level 3; L2, Level 2; L1, Level 1; MLU, Midwife led unit; PNT, Postnatal transport

Table 3: Referring and receiving neonatal unit levels for all postnatally transported infants 23^{+0} to 27^{+6} weeks' gestational age within 72 hours of birth in the UK between 2011 and 2016. Taken from Paper 1

Chapter 3. Risk of severe intraventricular haemorrhage in the first week of life in preterm infants transported before 72 hours of age

Centralisation of neonatal care has led to reduced mortality in extremely preterm infants (11), but is associated with an increased risk of IVH (23, 24, 53). However, studies reporting this association did not differentiate between early perinatal brain injury occurring within the first week of life and later brain injury. The use of early CrUSS within the first 7 days of life as an outcome measure is imperative to correlate early perinatal events to brain injury, which in these studies could otherwise have occurred due to later postnatal event, such as sepsis. To allow a better understanding of the relationship between early PNT and perinatal brain injury, I evaluated the risk of severe IVH in preterm infants transported within the first 72 hours of life whilst adjusting for key antenatal (including ANS) and perinatal confounding factors.

For statistical analysis, the study population was grouped according to transport status, then further sub-grouped into <28 week and 28-32 week GA cohorts. Associations between demographic and clinical variables with transport and with IVH were assessed using chi-square tests and Mann-Whitney-U test. Multivariable logistic regression was used to calculate the adjusted odds ratio for the association between transport and IVH. This methodology was used as opposed to propensity score matching to control for confounding factors, as the relatively small study population within subgroups analyses, along with the imbalance of covariates between groups would result in sample attrition potentially leaving too few cases for meaningful analysis with the latter method. Furthermore, confounders considered for inclusion within in the model included those which occurred after infant transportation (e.g. inotrope).

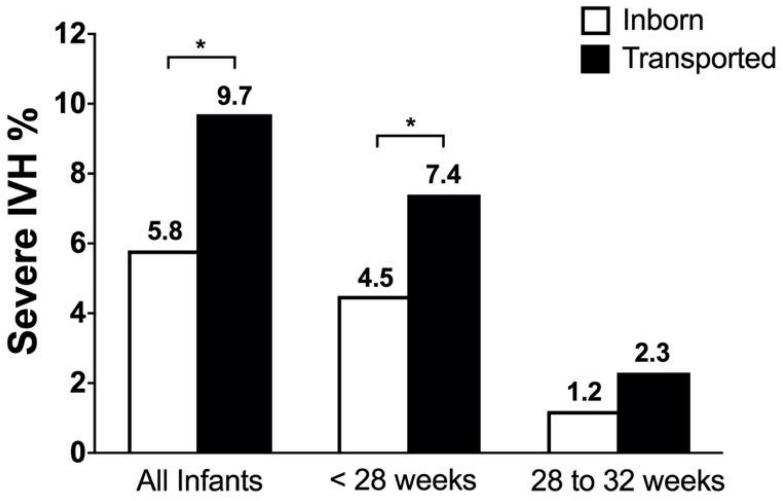
My findings have shown early PNT is associated with an increased risk of severe IVH in extremely preterm infants compared to infants who are born and remained at a level 3 hospital (52). This association remained despite adjusting for major confounding factors. Although, there was no significant difference in the risk of severe IVH for transported infants who were 28 to 32 weeks' GA (figure 11), this may be due to these infants having an overall lower risk of IVH in this GA subgroup. My results are consistent with previous studies demonstrating an increased risk of severe IVH in transported preterm infants (23, 24, 53). However, through evaluation of CrUSS on day 7 of life I was able to demonstrate association with early perinatal brain injury. This is a key period for the development of IVH (22, 35) and this raises the possibility that the transportation process itself contributes to this additional morbidity, particularly as other key risk factors for IVH were controlled for. Though I acknowledge some residual confounding effect may remain due to variable data entry error or imprecision and unmeasurable variables, such as differences in provision of care. Additionally, utilising day 7 CrUSS for outcome analysis allowed for any inflammatory process related to the transportation process to evolve but minimised the effects related to later postnatal insults. The mechanism for the association between IVH and early transport is likely multifactorial and not fully understood. It is plausible the noxious stimuli infants are exposed to during the transport pathway (88, 89, 100, 174), as previously described, could contribute to the increased risk of brain injury seen in these infants. These noxious stimuli are associated with fluctuations in heart rate, respiratory rate and blood pressure in preterm infants, which can ultimately result in changes in the cerebral vasculature (175, 176). Hoffman *et al* (31) demonstrated preterm infants who developed severe IVH had increased time with impaired cerebral autoregulation during the first 96 hours of life

compared to infants without IVH. Moreover, exposure to excessive WBV can cause morbidity in adults including neurovascular injury and capillary wall damage in animal models. This cellular injury could, in part, contribute to poor neurological outcomes in extremely preterm infants. CrUSS is an effective imaging modality for the evaluation of gross neuropathology but it does not allow for detection of more subtle changes on a microscopic or molecular level. Based on my findings in this study, further research to investigate the mechanism by which these stimuli contribute to brain injury is essential. This will allow development of preventative measures and aim to reduce poor neurological outcomes.

A complete course of ANS prior to delivery is associated with a significant reduced risk of IVH (both mild and severe) in preterm infants <32 weeks GA irrespective of transport status (32, 52). However, transported infants were significantly less likely to receive a full course of ANS (52). This is a measure of the quality care reported by the National Neonatal Audit Programme and remains an area of on-going improvement within neonatal networks (1). The neuroprotective effect of ANSs is seen with extremely preterm infants undergoing early PNT, although those receiving a full course of ANS still had an increased risk of severe IVH. However, a full course of ANS was still beneficial over having either no or an incomplete course (figure 12). The reduction in risk of severe IVH seen in these infants who received a full course of ANS, could be related to the upregulation of GFAP resulting in less fragility of the germinal matrix (30) or lung maturation leading to reduced respiratory distress and need for invasive ventilation (7). IVH remains one of the leading causes of severe neurodisability in preterm infants. I have demonstrated that the risk of severe IVH associated with early PNT in preterm infants is not completely mitigated by ANSs.

Magnesium sulphate is an additional antenatal neuroprotective agent that has been shown to substantially reduce the risk of cerebral palsy (6) in childhood. However, the use of magnesium sulphate was not recorded within BadgerNet during the study period and therefore, could not be included as a potential confounding factor within the model. Although, it is unlikely to affect the prevalence of early IVH (177, 178).

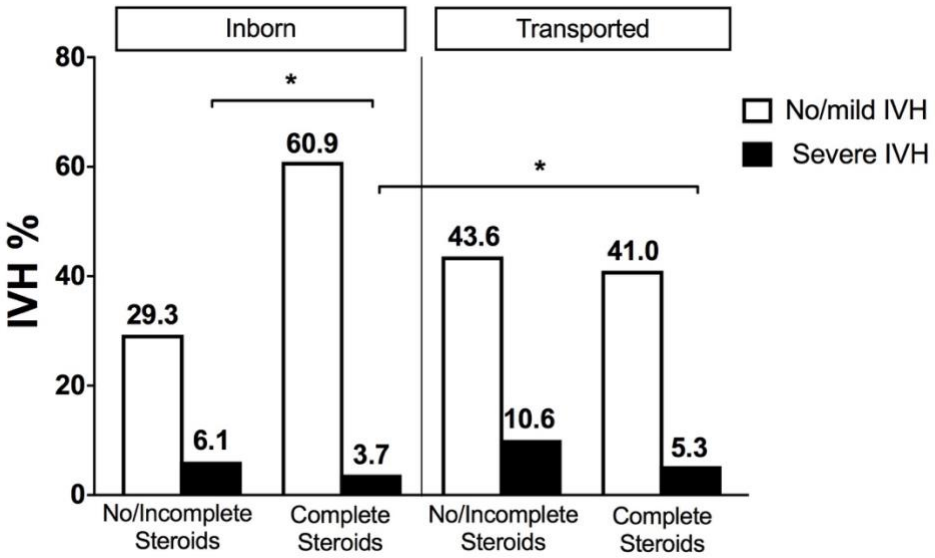
Overall, my analysis provides valuable information for obstetricians and neonatologists of the need for on-going service development to facilitate IUT with the aim to reduce adverse outcomes associated with PNT. However, IUT is not always possible, therefore potential contributing factors to these associated risks need to be explored. Improvements in ventilation, incubator thermoregulation and reduction in exposure to noise and WBV could help minimise physiological instability and risk of neurological insult in these high-risk infants.



Severe IVH, Grade 3 and 4

Figure 11: Comparison of proportion of severe intraventricular haemorrhage (IVH) between transported and inborn infants by gestation subgroups.

*** Denotes significance, p<0.05. Taken from Shipley *et al* (52).**



No/Mild, None/Grade 1 and 2; Severe IVH, Grade 3 and 4

Figure 12: Comparison of proportion of no/mild intraventricular haemorrhage (IVH) and severe IVH in inborn and transported infants less than 28 weeks' gestational age, sub grouped by antenatal steroid course. * Denotes significance, p<0.05.

Taken from Shipley *et al* (52).

Chapter 4. Quantifying the impact of centralised neonatal care on the family: A national population study

Parents of preterm infants are faced with a multitude of stressors, from the anxiety of unexpectedly giving birth early, concerns regarding risk of mortality through to long-term survival with severe co-morbidities. Consequently, they have been shown to be at an increased risk of poor mental health compared to parents of term infants (40, 45). Centralisation of care is essential to improve survival for extremely preterm infants (11), but may result in an infant being cared for in a centre located further away from home (Paper 3). This can add additional stress due to separation from children and their support network, the burden of travelling long distances and associated financial implications (car parking, sustenance and travel costs) (57). The charity Bliss recently reported a large number of level 3 centres had inadequate accommodation, car parking provision or ability to provide financial support (58). However, there is a lack of quantification of key factors that contribute to this additional stress placed on parents through centralised care, a requirement to support adequate funding and improve provision of services. Furthermore, these aspects are particularly important if an infant were to die whilst away from their BH, as parents are likely to be isolated and away from their support network. Currently, there are considerable gaps in the provision of mental health professionals on neonatal units (46) and an absence of studies to quantify the proportion of high-risk parents who are more likely to require these services. To gain a better appreciation of these potential stressors, I evaluated the time and distance an infant is cared for outside of their BH, early maternal-infant separation and the number of infants who die whilst away from their original BH.

Centralised neonatal care resulted in 58.6% of all extremely preterm infants admitted to neonatal units in the UK being transferred and cared from away from their BH, with almost a third requiring at least two care episodes in a non-BH (Figure 13)(Paper 3).

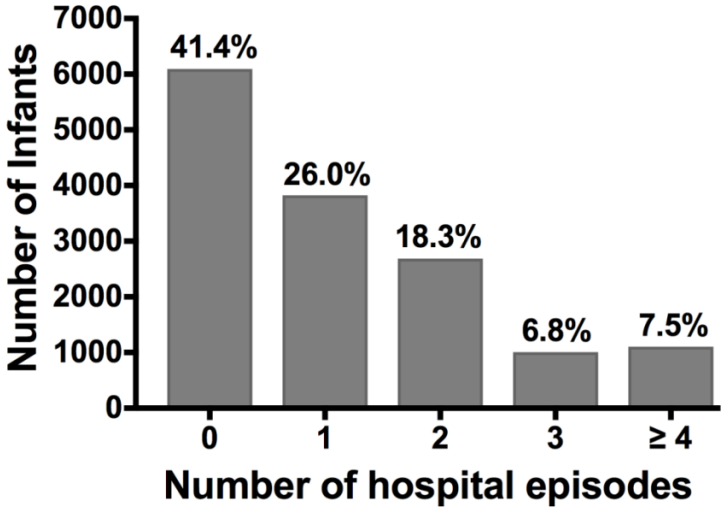


Figure 13. Number of hospital episodes for infants cared for away from their booking hospital during neonatal care. Taken from Paper 3.

Early PNT occurred in 19% of these infants, a period during which maternal-infant separation is likely to occur secondary to maternal health concerns post-delivery, lack of maternal inpatient bed or inability to travel within the same ambulance as her newborn (55). This maternal-infant separation not only affects bonding (55) and maternal mental health outcomes (40) but often results in a barrier to the provision of colostrum and maternal breastmilk (55), which is beneficial to infants to reduce the risk of necrotising enterocolitis (179), late onset sepsis (180) and improved neurodevelopmental outcomes (181). Long-term physical separation and lack of hospital accommodation, particularly if an

infant is being cared for a long distance from their home, over the course of an infant's hospital admission can also affect the transition to direct breastfeeding (55). IUT would mitigate the negative impact associated with early maternal-infant separation, as well as reducing the risk of severe brain injury (15, 182). However, there are substantial barriers to undertaking IUTs in the UK (19), which potentially has contributed to a significant decrease in IUTs over time (Paper 1). Formal evidence-based guidance to aid clinicians in the decision of who and when to IUT could prevent delayed referral. Modifications in service provision to ensure designated staff to undertake IUT and adequate staffing at level 3 maternity units to manage these additional women could also help improve the number of IUTS. In light of previous work (19, 161) and my data, the benefits and cost of a co-ordinated national IUT pathway and service needs further consideration. Nevertheless, it is important to recognise that IUT still requires an infant to be cared for away from their BH and is likely to contribute to additional parental stress. This is supported by Porcellato *et al* (167), who found through structured parental interviews the lack of familial support, logistical issues around child care and financial impact made IUT a disruptive and anxiety provoking experience.

Infants were cared for away from their BH a median of 39 days throughout their hospital admission, with almost a third spending more than 60 days away (Figure 14). This was particularly true for 24 and 25 weeks' GA infants. This finding is in keeping with Seaton *et al* (183) who found 24 to 25 weeks' GA infants had an overall longer length of hospital stay then those at 26 to 28 weeks. Moreover, evaluation of infants at 23 and 24 weeks' GA showed a large cohort had relatively short lengths of stays. This is likely to represents infants who died early in their admission with the survivors requiring a subsequent prolonged length of stay away (Figure 14). These data support a better understanding of

the length of time away from a BH providing valuable information for planning resources, service configuration and parent counselling.

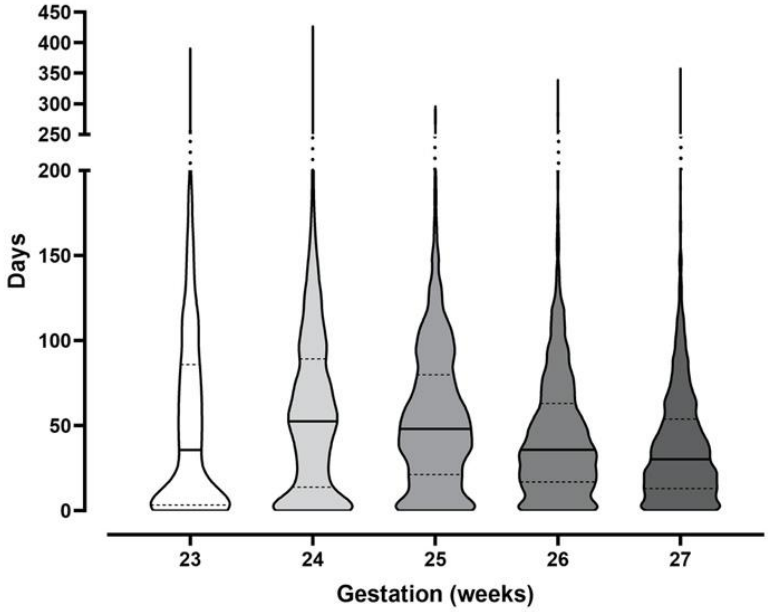


Figure 14. Violin plots (median and IQR) of the number of days transferred infants spent away from their booking hospital stratified by gestational age. Taken from Paper 3.

Infants were typically cared for an average return distance of 74km (46 miles) from their parent's residence. This could equate to 518km (324 miles) per week if visiting every day and highlights a substantial barrier if parents are unable to drive, in addition to the cost and travelling time (Figure 15). This additional burden is exacerbated by the shortage of available parent accommodation at neonatal units and the inability to provide financial support (58). Overall, half of mothers of infants cared for away from their BH had a previous pregnancy suggesting these parents may have other children at home (Paper 3). Given the combined length of time an infant spends away from a BH and distance from parents' residence to a non-BH, it is likely parents will be faced with the difficult decision between

spending time caring for other children and their sick infant on the neonatal unit. This can have an effect on bonding and long-term family functioning (184).

Half of extremely premature infants who died during this period did so away from their BH. This represents a large cohort of families who are likely to be away from their support network at a time of heightened stress. Lack of social support following a neonatal death is associated with an increased risk of adverse grief reaction (185). This highlights the essential need for provision of mental health workers and accessible bereavement support with subsequent follow up, which maybe challenging due to the distance from home, to reduce long-term adverse psychological outcomes.

Overall, the results presented in this thesis demonstrate that there is an additional burden on families of extremely preterm infants who are transferred away from their BH with early maternal-infant separation, extended periods away from home and the need to travel long distances. There is a further impact on parents of infants who die away from their BH due to lack of bereavement support from family and access to services at a non-BH. Service providers need to focus on improving the care we deliver for these families by minimising separation, provision of adequate facilities, financial support and access to psychological services to improve parental well-being and mental health.

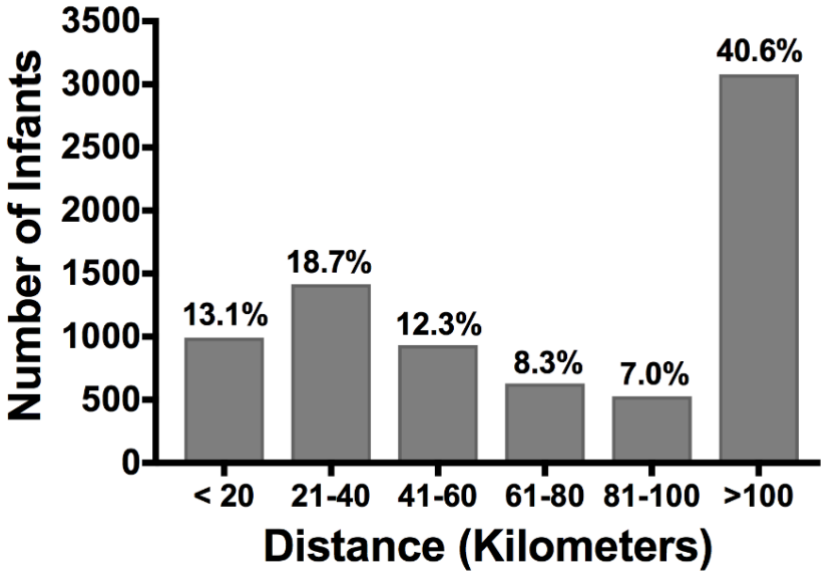


Figure 15. Return distance from the mother's place of residence to non-booking hospital.

Taken form Paper 3

Chapter 5. Trends in the prevalence and management of hypoxic-ischaemic encephalopathy in the therapeutic hypothermia era: a national population study

HIE remains the largest contributor to brain injury in the UK (47). TH is now the standard of care for moderate/severe HIE in the UK following evidence supporting reduced mortality and severe neurodisability (66, 67, 69, 70, 186). Despite this, recent data on the current management and outcomes of infants with HIE in the TH era are lacking.

TH has been demonstrated as a safe and acceptable treatment with minimal adverse effects for infants ≥36 weeks' GA (78, 187). However, there have been reports of a therapeutic drift to management of infants, such as those with mild HIE and late preterms, outside of current evidence-based guidance (78, 91, 93, 94). Although infants with mild HIE have adverse outcomes at 18 months (death or moderate or severe disability), there is insufficient evidence to recommend routine use of TH (93). Similarly, the use of TH in late preterm infants has been associated with higher rate of mortality and complications compared to more mature infants (78, 91). To gain a better understanding of the current trends in HIE and management of infants outside of current guidance, I examined the prevalence of mild, moderate and severe HIE in both term and late preterm infants within the UK population. For statistical analysis, the study population and subgroups (late preterm and mild HIE infant) were divided into two equal epochs to evaluate temporal changes. Associations between demographic and clinical variables for infants with moderate/severe HIE were assessed using chi-square test and Mann-Whitney U test.

My study identified 12,195 infants with a diagnosis of HIE between 2011 - 2016. The prevalence of moderate/severe HIE within the population was 2.03 per 1000, which is similar to the findings of previous studies (47, 59). Both the prevalence of moderate/severe HIE (2.00 vs 2.07 per 1000, p=0.12) and overall mortality of infants 34 to 42 week' GA with moderate/severe HIE (0.21 vs 0.20 per 1000, p=0.32) have not significantly decreased over time, highlighting HIE remains a major cause of mortality and severe neurodisability in the UK (Paper 4). Following the publication of the TOBY trial in 2008 (66), the implementation of TH as a treatment for HIE in infants $\geq$36 weeks GA has significantly increased (1.09 vs 1.40 per 1000, p<0.001) with a corresponding decrease in mortality (15.1% vs 10.9%, p<0.001) (Paper 4). Reassuringly, mortality in these infants treated with TH has continued to decrease since the TOBY study from 20% to 12.7% (78)(Paper 4), which is likely to reflect a change in practice recognising and implementing treatment earlier, as well as improving intensive care management.

There has been no temporal change in the prevalence of mild HIE and overall mortality rates were low. Nevertheless, the proportion of infants managed with TH has significantly increased with almost a third of infants being treated (Paper 4)(93, 94). The extension to treating these infants could be potentially on compassionate grounds or on the background of increasing evidence that infants with mild HIE may have adverse outcomes of death or moderate/severe disability at 18 months of age (93, 188). Similarly, my study, which is the largest to date to evaluate the rate of HIE and use of TH in late preterm infants within the UK population, has demonstrated the prevalence of moderate/severe HIE and rate of mortality has remained unchanged over time, but the use of TH has significantly increased

(26.0% vs 39.4%, p<0.001) (Paper 4). Moreover, late preterm infants with moderate/severe HIE had a significantly higher rate of mortality compared to more mature infants (Figure 16) (Paper 4)(91), which highlights the need for further studies to evaluate the safety and efficacy of TH in these infants.

Overall, these additional infants who undergo treatment with TH outside of the recommended guidelines could result in an increased number of infants being separated from their parents for the duration of the therapy. This risks additional strain on already overwhelmed transportation teams (86), adding to cot capacity issues (46), increasing healthcare costs (189) and exacerbating the psychological impact on the family whilst their infant is being cared for away from their BH (45, 184). These infants may also be exposed to the unnecessary risks from TH management (187) and invasive procedures, which can further add to their comorbidity. Before widespread TH occurs in these infants, studies are required to evaluate its efficacy in both late preterm infants and mild HIE to establish any benefits (or harm) and avoid transportation of these infants to another centre for treatment.

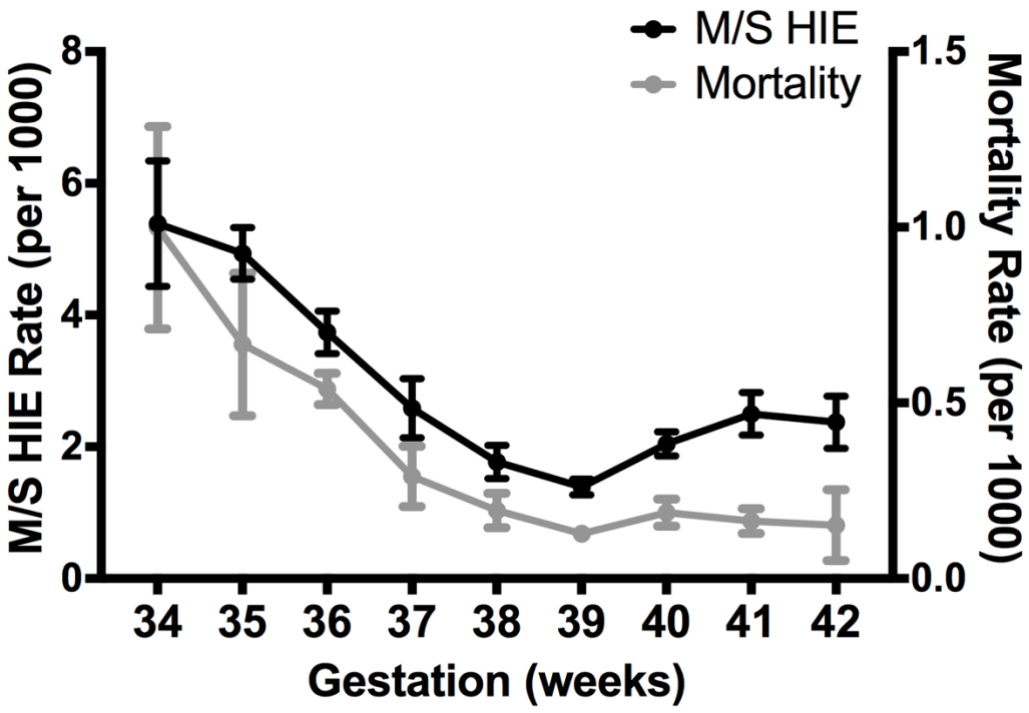


Figure 16. Rate of infants with moderate or severe hypoxic-ischaemic encephalopathy managed with therapeutic hypothermia and mortality for infants born in the UK, between 2011-2016, by gestational age. Taken from Paper 4.

Chapter 6. Association of birth in centres without active therapeutic hypothermia and seizure-free survival following neonatal hypoxic ischaemic encephalopathy: A nationwide study

Active TH has been successfully established in the UK within specialist cooling centres. Infants born in non-cooling centres are usually passively cooled prior to transportation by designated neonatal transport teams. This pathway for those born in a non-cooling centre could lead to adverse events and delay in treatment with active TH, as previously discussed. Delays in treatment beyond 6 hours of age could reduce potential benefits of TH, particularly as there is evidence to suggest early treatment with TH is associated with better neurodevelopmental outcomes (75, 76). However, these studies where limited by sample size and there have been no studies assessing the impact of a nationwide approach to centralised TH for infants with HIE. I therefore explored the relationship between birth in a non-cooling centre without immediate access to TH with short-term outcomes.

For statistical analysis, clinical variables between infants born in non-cooling centres and cooling centres were compared using chi-squared test for categorical data and Mann-Whitney U test for non-normally distributed data. The effect of birth in a cooling centre on outcomes was described using odd ratios. Propensity score matching was used as, similar to randomised control trials, this methodology allows separation of study design and outcomes. The large sample size in this study resulted in a good untreated-to- treated ratio. This permitted, through matching, creation of balanced groups of demographic and clinical covariates prior to analysis. Furthermore, unlike regression, propensity score methods result in estimation of marginal treatment (population-average) effects.

In the UK, 46.7% of infants ≥36 weeks GA with moderate/severe HIE managed with TH were born in a non-cooling centre. These infants were significantly less likely to survive without seizures, a result that was likely driven by the significantly higher seizure rate as the rates of mortality were similar regardless of birth centre (Paper 5). Although I was not able to establish long-term neurodevelopmental outcomes, previous studies have shown infants with HIE who develop seizures have a worse long-term neurological outcome compared to those with no seizures (62, 64, 65).

My findings are consistent with Natarajan *et al* (190) who demonstrated no significant increase in mortality based on place of birth, but this study did not find any difference in severe neurodisability. However, this study was limited by small sample size (n=205 infants compared to n=5059 in my study) and differences in key baseline characteristics. Using propensity score-matching, I was able to create well balanced groups for major confounding factors including the grade of HIE. There are likely several contributing factors for this association between birth in a non-cooling centre and significantly increased risk of seizures in surviving infants. Although, my study population was well matched for key background and confounding factors, I was unable to account for unmeasured confounders, such as effectiveness of resuscitation, quality of intrapartum and early neonatal management. There has been concern that centralisation of neonatal care has led to de-skilling of staff (191) and it is plausible that sub-optimal management within non-tertiary centres in part contributes to this increased morbidity. However, on evaluation of infants born in level 2 centres only, who are likely to have experienced similar perinatal care, the association of birth in a cooling centre and improved neurological outcomes persisted. This suggests the cause is less likely to be due to differences in early care alone (Paper 5).

Another consistent finding and potential contributor to the additional seizures seen in these infants is the delay in reaching optimal therapeutic temperature (Figure 17)(Paper 5) (80, 85). It is plausible that infants born in a non-cooling centre receive only partial protection of TH through either delayed or ineffective passive cooling resulting in decreased dampening of excitatory neurotransmitters and apoptosis (75). I found a significant reduction in seizures in infants with immediate access to TH within a cooling centre, supporting the hypothesis that additional neuroprotection is provided by early cooling. Evaluation of the benefits of early versus late cooling is not possible with this database, a greater understanding of this is essential to improve long-term outcomes.

In addition to potential differences in early care and delay to TH, infants born in a non-cooling centre must undergo early PNT for further management. The transport environment exposes infants to potentially detrimental environmental factors (88, 89, 100, 102, 103). It is possible that the underlying inflammatory and cell death pathways seen in infants with HIE are exacerbated by exposure to these stimuli resulting in worse neurological outcomes. Future studies should be undertaken to evaluate the effect of excessive vibration exposure, which has been shown to contribute to brain injury in animal studies (118), on the more mature infant brain and those with hypoxic brain injury. This would allow the investigation of potential neuroprotective agents, which could be used as adjuvants to TH and potentially improve outcomes in these infants. Furthermore, evaluation of physiological parameters and biomarkers of brain injury, such as urinary S100B during ambulance transportation could allow correlation between level of exposure and potential brain injury. This will aid development of strategies to minimise noise and vibration exposure.

a)

		Age at Admission		
		<6 Hours	6-12 Hours	>12 Hours
Temperature	>34°C	4.2% (n=86)	11.9% (n=242)	2.4% (n=48)
	33-34°C	12.7% (n=259)	48.3% (n=979)	6.1% (n=124)
	<33°C	2.5% (n=52)	10.3% (n=209)	1.3% (n=27)

b)

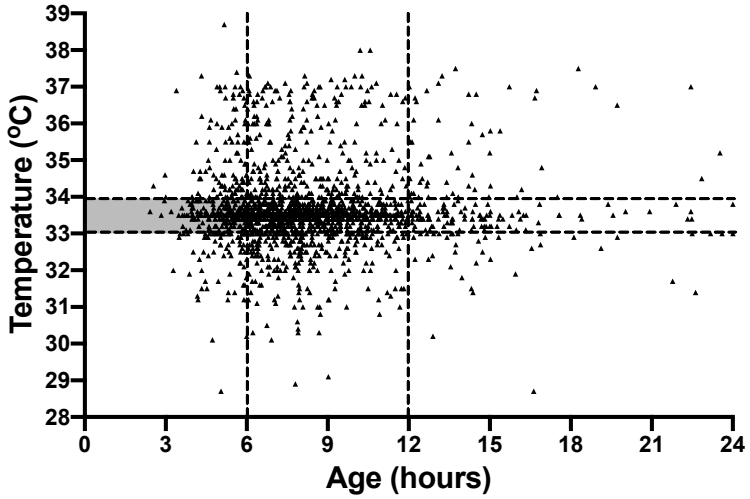


Figure 17. Admission temperature and arrival time following transfer to a cooling centre (n = 2027) (a) tabular data (percentage) on each of the categories based on admission temperature and time, (b) individual plots for each infant divided into key zones as in table (a) with the shaded area as optimal temperature in the 6 hour therapeutic window for infants ≥36 weeks gestational age with moderate or severe hypoxic-ischaemic encephalopathy. Taken from Paper 5.

Chapter 7. The National Neonatal Research Database

The use of large clinical databases of routinely collected prospective data for research has increased in recent years (192). These data can be used to evaluate trends within a population, provide insight into causes and outcomes of disease, compare population outcomes between interventions and develop risk factor models (193). In addition, the use of routinely collected electronic data in clinical trials has been shown to reduce the costs associated with paper based data collection (Case Record Forms) (194).

Data from the NNRD has been used to evaluate outcomes for all the drafted papers contained within this thesis. The NNRD holds demographic and antenatal details, along with prospectively recorded daily clinical data and outcomes from every infant admitted to UK neonatal units, with the exception of Northern Ireland. The data is anonymised, cleaned prior to entry into the database, screened for erroneous entries and amended by the Neonatal Data Analysis Unit, in addition to external checks by clinicians for key data items.

7.1. Strengths and Limitations

The main strength of the NNRD is the large number of neonatal patients contained within the database from 180 neonatal units. This has provided a reliable representation of the population of infants admitted to neonatal units nationwide, minimised variation by geographical location and in clinical practice between units, allowed population trend analysis over time and permitted adequately powered statistical analyses. Battersby *et al* (195) and Jawad *et al* (196) evaluated the data quality within the NNRD and found completeness of data for common baseline data items (e.g. gender, GA, birthweight) that

have been reported across large UK neonatal trials was high (>95% complete). Hence, validating the use of this database for research purposes.

However, there are several limitations associated with the NNRD:

- Northern Ireland neonatal units currently do not contribute to the database. On account of this, the results of my study cannot completely describe population trends across the entire UK.
- The number of contributing databases has increased over time since establishment of the database in 2007. The NNRD included all neonatal units in England by 2012, and all units in Wales and Scotland by 2015. Data were collected for all the previously discussed studies between 2011 to 2016. In 2011, 90% of neonatal units in England contributed to the NNRD. Despite this, I was able to use a standardised extrapolation method (47) to give a lower and upper estimate of missing data using actual data from admissions and outcome of interest rates from 2012-2016.
- It was not possible to accurately determine missing data from absence of a characteristic for some dichromatous data items (e.g. early risk of infection, preeclampsia).
- Data items can lack detail resulting in potential data inaccuracy. For example, the presence of seizures is a dichromatous outcome within the database. Based on NNRD data alone, it is not possible to determine between electrical and clinical seizures. Similarly, in 2016 the NNRD included additional detail to the TH data field (passive and active cooling). However, I was unable to use this additional data within my studies as this was either unavailable prior to 2016 or poorly completed. Therefore, it is unclear from NNRD data whether infants recorded as undergoing cooling within the database underwent passive or active cooling. This potentially could have impacted on the proportion of late preterm infants or those with

mild HIE recorded as undergoing TH within Paper 4. Although, this is less likely as only those cooled for a period > 24 hours were included.

- Missing data. Jawad *et al* (196) reported high completeness for baseline data variables commonly found within large UK trials. However, other data items had between 10 to 30% missing data. The data variables used for my studies had between 0.1 to 15.7% missing observations, which could lead to loss of precision in the estimation of treatment effect. For this reason, I have used multiple imputation or separate categories to minimise bias resulting from missing data.

- Erroneous data. The Neonatal Data Analysis Unit screens for erroneous data, although this cannot mitigate completely if the data is incorrect at the point of entry.

- The use of magnesium sulphate, an important neuroprotective agent, was not recorded within the NNRD until after 2016. Therefore, I was unable to include this as a confounding factor in Paper 2. Future studies should consider inclusion of this variable in models that evaluate neurological outcomes in preterm infants.

- Long term outcomes are not universally recorded within the database, therefore limiting my study outcomes to the point of discharge. Likewise, the NNRD lacks neuroimaging data, which would allow better evaluation of neurological outcomes.

- Infants who die in the delivery room or do not require medical intervention are not entered on the database. On account of this, national ONS data that includes all live births in England and Wales were used to assess population prevalence and mortality rates in my study (Paper 4)(222).

Chapter 8. The effects of whole body vibration, as experienced during neonatal ambulance transportation, on the developing brain

8.1. Background

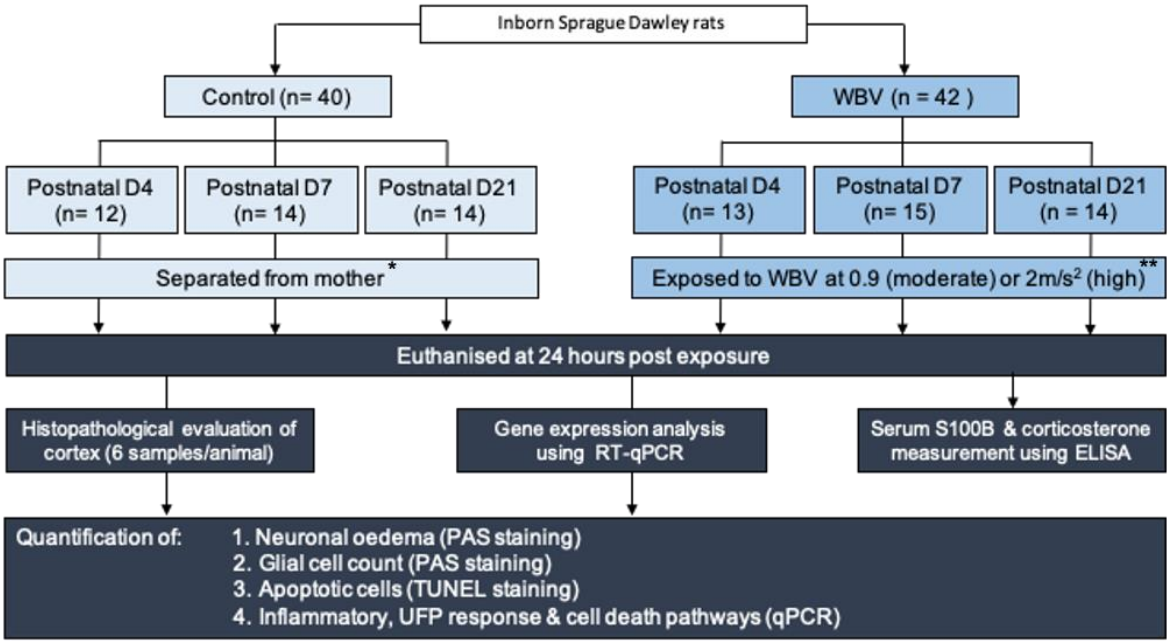
Excessive WBV exposure from motor vehicle use has been associated with detrimental health outcomes in adults (109, 110, 114, 115). The exact mechanism of injury secondary to WBV exposure is not yet fully understood but is likely to result from twisting and shearing forces within the tissues of the body from transmitted vibration waves. Animal studies have demonstrated this tissue damage subsequently leads to activation of the inflammatory cascade in an attempt to restore cellular homeostasis and repair damage (118, 120). However, the often excessive inflammatory process can lead to cell death and can contribute to adverse long-term outcomes (134). These studies have mainly focused on the effects of WBV exposure in adult rodents, as well as the effects of chronic exposure rather than a single acute exposure episode. One hypothesis in this thesis is centralisation of neonatal care leads to an increased risk of early brain injury in preterm infants who undergo early PNT as part of their care pathway. Previous studies have confirmed that these high-risk infants are exposed to excessive WBV during ambulance transportation (88, 89, 101, 102) and have an increased risk of severe IVH (52, 53). Therefore, it is possible this excessive vibration through either direct injury as described above, or through impact of the brain against the cranium leads, to inflammation and cell death. At present, there is a paucity of evidence to evaluate the effect of short-term WBV on the preterm brain.

In addition to preterm infants, those with HIE and born outside of cooling centres also require early PNT (approximately 500-560 per year (13, 21)). This often occurs shortly after a hypoxic brain insult. Hypoxia of the brain tissue leads to the onset of inflammation, influx

of microglia to the area and subsequent apoptosis over the following hours to days (197). I have previously demonstrated that birth in a non-cooling centre without immediate access to active TH is associated with reduced survival without seizures (Paper 5). Seizure activity in these infants is reflection of ongoing secondary cell injury that occurs in these infants and correlates well with neurodevelopmental outcomes (64, 65, 198, 199). The underlying mechanism is likely multifactorial, but it is plausible that the transportation process could be a contributing factor. It is therefore important to evaluate the risk to this large cohort of high-risk infants who undergo early PNT secondary to centralisation care. Using my new rodent model of short-term WBV exposure, I evaluated the effect of this insult on microglial activation, inflammation and cell death pathways.

8.2. Methods

The methods used for evaluation of the effects of short-term WBV on the cortex of the brain using my neonatal rodent model are summarised in Figure 18. A more detailed account of the study methodology is given in Appendix 1.



WBV, Whole body vibration; D4, Day 4; D7, Day 7; D21, Day 21; m/s^2, metres per second squared; RT-qPCR, Real-time quantitative polymerase chain reaction; ELISA, Enzyme-linked immunosorbent assay; PAS, Periodic-acid Schiff; TUNEL, deoxynucleotidyl transferase-mediated dUTP nick end labelling; UPR, Unfolded protein response

* separated for 86 minutes (moderate intensity cohort) or 90 minutes (high intensity cohort)

** exposure time of 86 minutes (moderate intensity cohort) and 90 minutes (high intensity cohort)

Figure 18: Summary diagram demonstrating the study outline for the evaluation of the effects of short-term whole body vibration on the developing brain

Due to guiding principles (three R's) underpinning the humane use of animals in research, different gender of animals were used for the moderate and high WBV groups. This has eliminated within group bias, but potentially could introduce sex as a confounding factor between vibration groups. Schwarz *et al* (200) found that male postnatal day 4 rodents have significantly more microglia in the cortex than females. This finding decreases with age. This could suggest the difference between moderate and high WBV groups is potentially greater than observed due to confounding by sex. However, they found no sex difference in the expression of pro-inflammatory cytokines (IL-1β, IL-6, TNFα) in the brain cortex of rodents at day 4 or at day 30. Consequently, evaluation of pro-inflammatory gene expression is less likely to affected by gender bias.

8.3. Results: The impact of WBV on the developing brain

Following development of a novel rodent model, I used D4, D7 (immature) and D21 (mature) rodents to evaluate the potential effects of a single short-term WBV exposure on the developing brain.

8.3.1. The Inflammatory Pathway

My study has shown exposure to a single period of short-term WBV resulted in a significant increase in reactive microglia in the cortex of the brain (Figure 19), similar to that seen with TBI. This response was reduced in D21 rodents compared to the more immature animals on postnatal D4 and D7 with a 1.2 fold increase in proportion of microglial present within the brain cortex of D21 animals compared to a 3.4 and 2.2 fold increase at D4 and D7, respectively (Figure 19). Furthermore, histological evaluation revealed significant neuronal oedema following exposure. These changes were evident in both postnatal D4 and D7 rodents, but to a lesser extent in the more mature rodents (Figure 20).

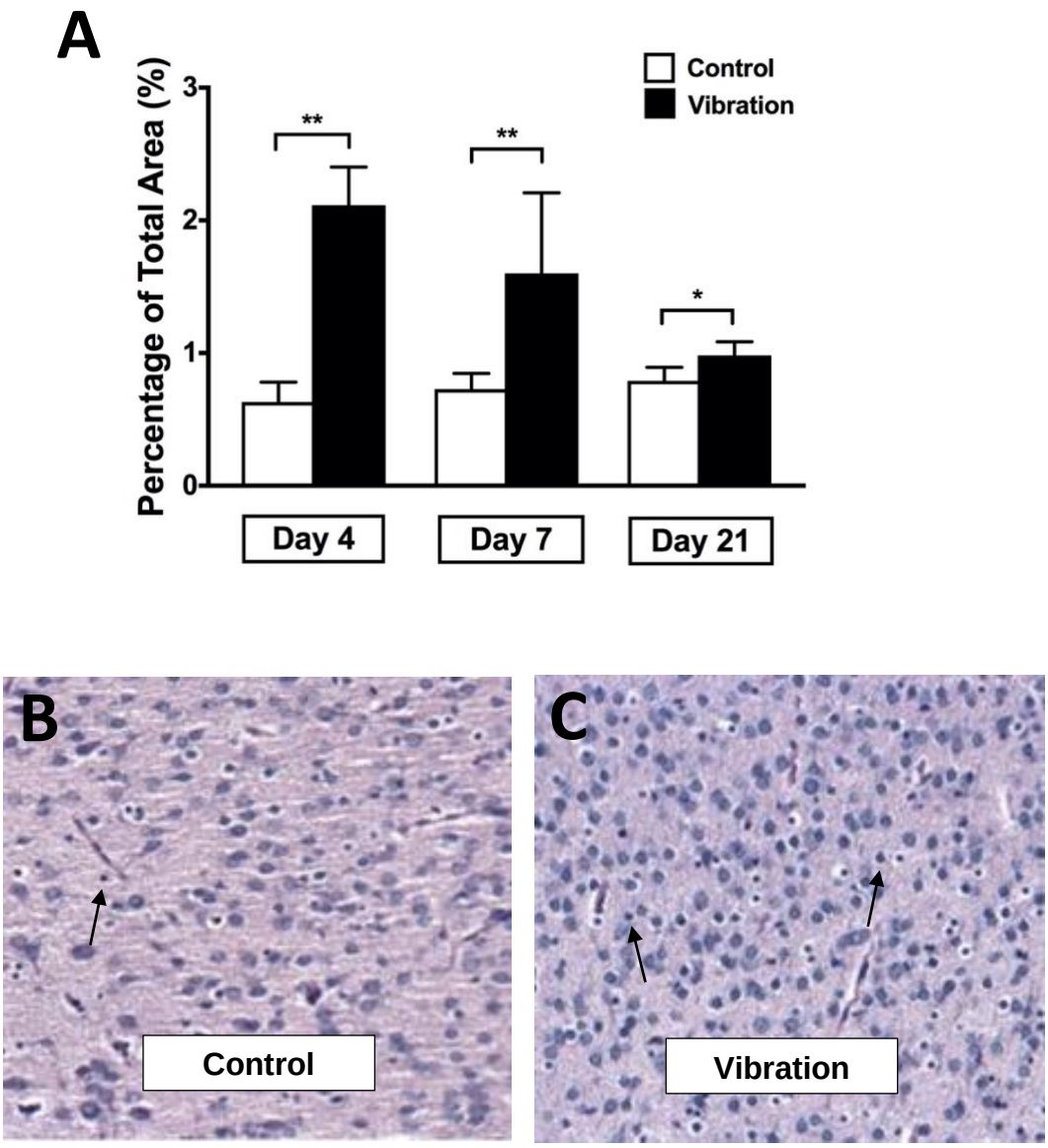


Figure 19. (A) Percentage of total area of microglia within the designated regions of interest in the brain cortex of control and vibrated rodents on postnatal day 4, 7 and 21 (n = 6-8/group). Representative slides of (B) microglia (arrow) in the brain cortex of control postnatal day 4 rodents, and (C) brain cortex of vibrated postnatal day 4 rodents demonstrating reactive microgliosis (arrows) (x20 magnification). Data represent mean ± SD * p = <0.05, ** p = <0.01.

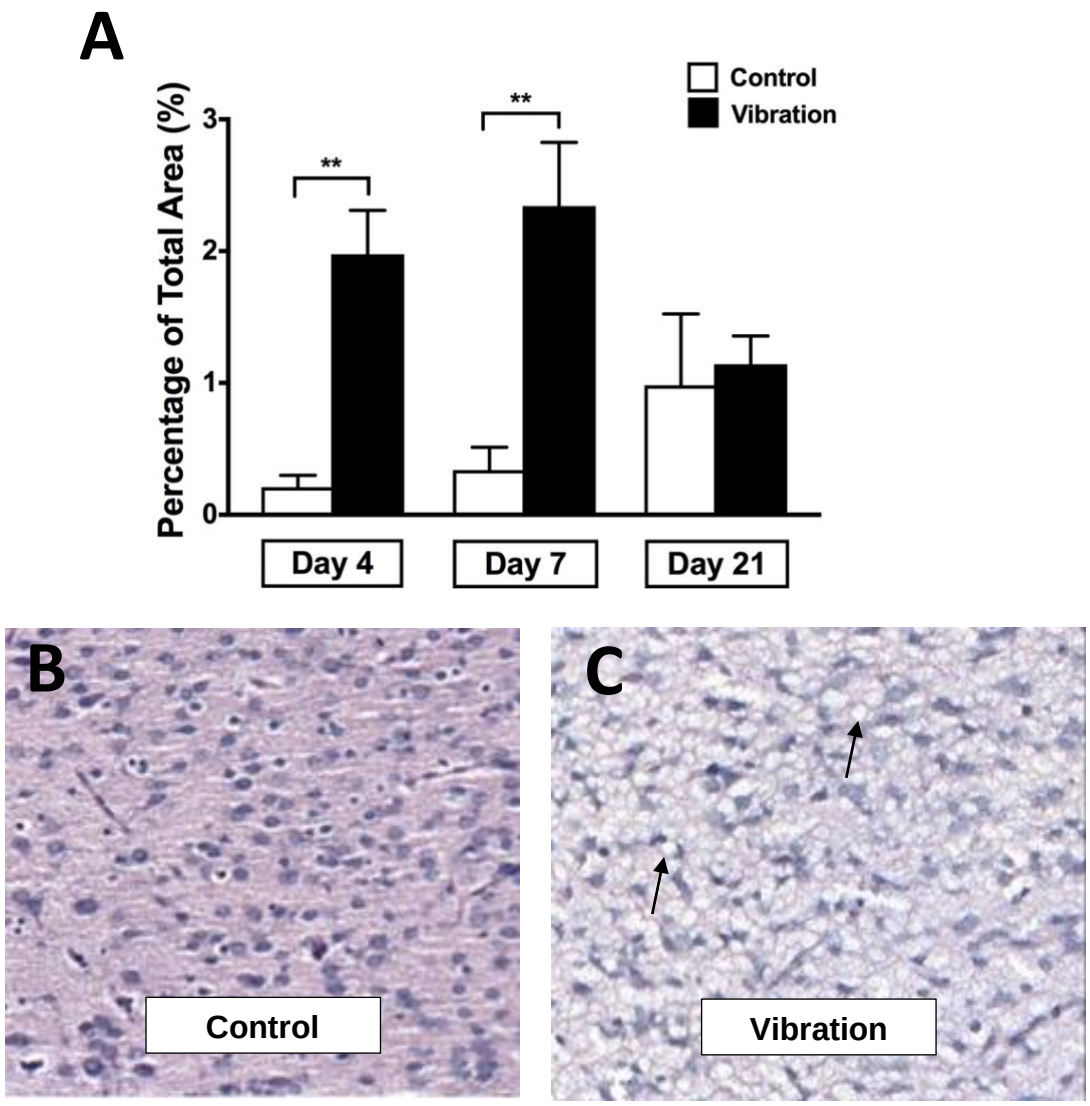


Figure 20. (A) Percentage of total area of neuronal oedema within the designated regions of interest in the brain cortex of control and vibrated rodents on postnatal day 4, 7 and 21 (n = 6-8/group). Representative slides of (B) the brain cortex of control postnatal day 4 rodents and (C) Brain cortex of vibrated postnatal day 4 rodents demonstrating neuronal oedema (arrows) (20x magnification). Data represent mean ± SD. ** p = <0.01.

These findings support the hypothesis that excessive short-term WBV induces primary pathological injury and activation of resident immune cells within the cortex. Parallel upregulation of TRL4 (Figure 21) within the cortex of immature rodents is likely to result in predominance of the M1 phenotype of microglial and drive subsequent release of pro-inflammatory cytokines. This is further supported by the increase in Il-6 and TNFα (Figure 21). Surprisingly, in my model, Il-1β, a pro-inflammatory cytokine implicated in the regulation of other cytokines, cell apoptosis and oedema formation, was not significantly increased following short-term WBV exposure in this group. This could be related to the timing of tissue sampling, as Il-1β levels peak 12 hours post injury and are directly related to the severity of the injury (123). This highlights the importance of further studies to evaluate the progressive effects of inflammatory changes within the brain tissue over time and correlation with neurological outcomes.

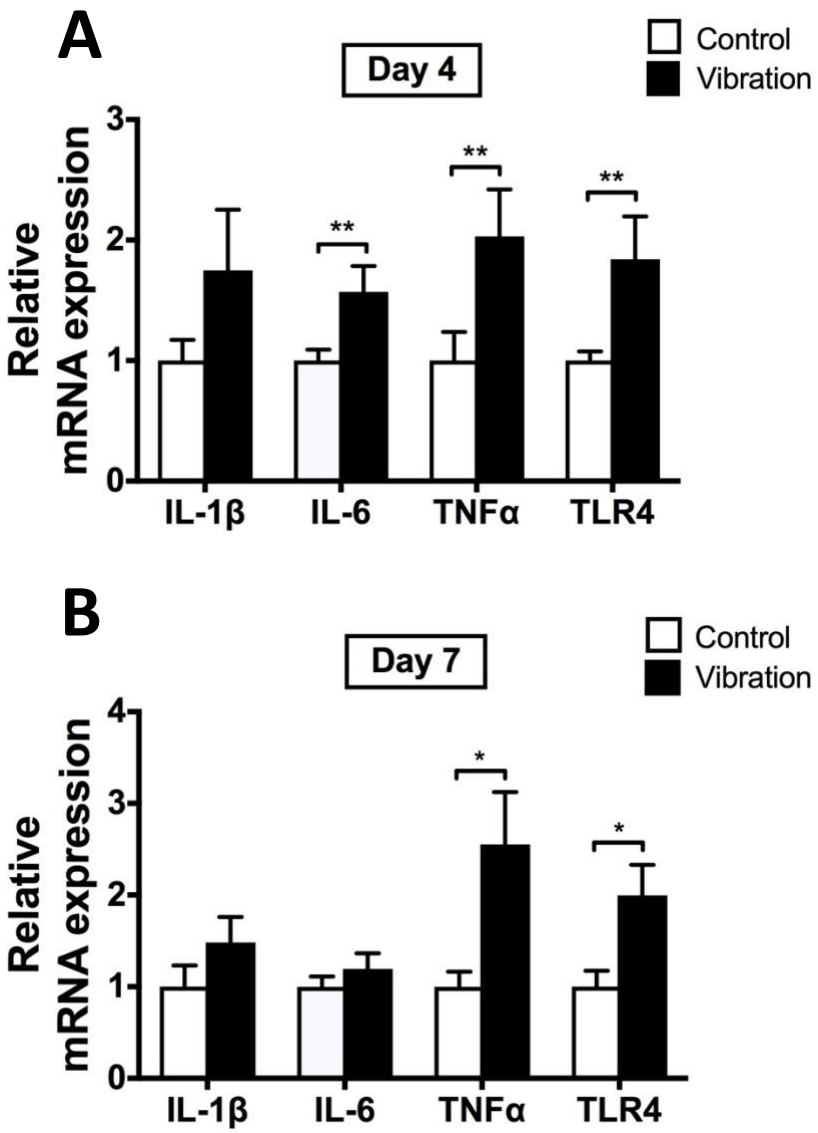


Figure 21. Relative mRNA expression of pro-inflammatory genes in the brain cortex of control and whole body vibration exposed animals on (A) postnatal day 4 and (B) postnatal day 7 (n = 8/group). The genes shown are Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), Tumour Necrosis Factor alpha (TNFα) and Toll-like receptor 4 (TLR4). Data is shown as mean ± SEM and is expressed as relative fold change compared to control animals. * p = <0.05, ** p = <0.01

In contrast, there was no increase in mRNA expression of pro-inflammatory genes (IL-1β, IL-6, TNF, TRL4) despite evidence of reactive microgliosis in D21 rodents (Figure 22). The lack of upregulation of pro-inflammatory genes in the more mature animals could be a reflection of less cell damage resulting in decreased release of ROS and endogenous factors (DAMPs). The subsequent lack of TLR-4 upregulation decreases microglia influx into the cortex and promotion of pro-inflammatory M1 phenotype microglia. On the basis of lack of evidence of a pro-inflammatory response, it is possible the increase in microglia seen in D21 rodents post insult are of the anti-inflammatory phenotype.

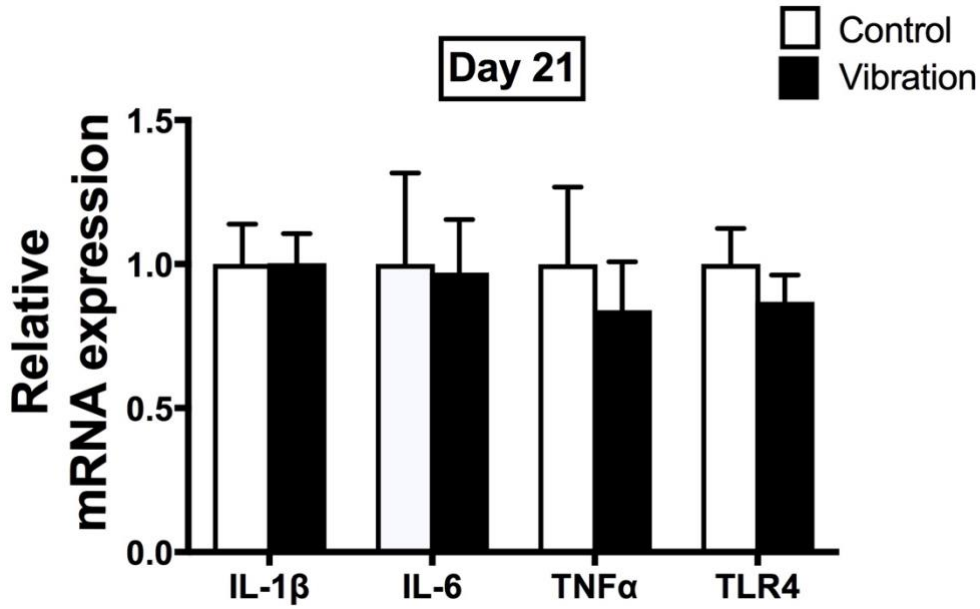


Figure 22. Relative mRNA expression of the pro-inflammatory genes Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), Tumour Necrosis Factor alpha (TNFα) and Toll-like receptor 4 (TLR4) in the brain cortex of control and whole body vibration exposed animals on postnatal day 21 (n = 8/group). Data is shown as mean ± SEM and is expressed as relative fold change compared to control animals.

The detection of IVH is easily visualised on CrUSS and correlates well with long-term neurological outcomes (34, 201). However, it has been suggested that CrUSS is unable to detect the entirety of white matter damage (201). Epidemiological studies have shown preterm infants who have had no detectable IVH on CrUSS still have worse neurodevelopmental outcomes compared to term infants (36), with almost a quarter of these infants developing moderate/severe neurosensory impairment (38). The findings in this thesis raise the possibility that the inflammatory process associated with short-term WBV, as experienced during ambulance transportation, could in part, contribute to the morbidity seen in infants with no detectable IVH and also potentially contribute to the development or extension of pre-existing IVH seen in infants following transportation (52). This theory is further supported by Supramanium *et al* (202), who found preterm infants with isolated GMH/IVH but no overt venous parenchymal infarct had increased microglial activation, cell apoptosis and TNFα expression in the periventricular white matter. Therefore, on this basis the persistent activation of the microglia and pro-inflammatory mediators could possibly be a contributing factor to white matter injury leading to long-term neurological deficits in infants exposed to WBV.

8.3.2. Endoplasmic reticulum stress, unfolded protein response and cell death pathways

Both prolonged ER stress and cell death have been associated with long-term adverse neurodevelopmental outcomes in both animal and human studies following TBI (203). Therefore, I investigated the effect of short-term WBV on ER stress and cell death pathways to explore the potential impact of WBV exposure on neurological outcomes.

ER stress has been demonstrated in the cortex of the brain of rodents exposed to TBI sufficient enough to cause mechanical cellular damage (136). Similarly, exposure to a single, short period of WBV equivalent to that experienced in an average neonatal transport resulted in ER stress within the more immature brain, potentially through direct cellular damage, as previously described. This was evident with a significant upregulation of GRP78 (Figure 23). GRP78 is a chaperone protein which ultimately leads to activation of the UPR in response to ER stress (204). Activation of the UPR through the PERK and IRE1 pathways attempts to maintain cellular homeostasis in response to this insult, as demonstrated by an increase in mRNA expression of both ATF6 and NFκB-1 (137, 205)(See Figure 7 for overview of UPR response and Figure 23). NFκB-1 can also be activated through several inflammatory signals (TNFα, IL-1β) and secondary to DNA damage. This transcription factor acts to limit cellular damage by contributing to the release of anti-inflammatory cytokines, promoting migration of microglia to the site of injury (205). This influx of microglia, although potentially beneficial through clearance of cell debris, can further exacerbate the inflammatory process within the cortex of the brain through release of pro-inflammatory cytokines, as previously described.

Following prolonged ER stress the UPR function is switched from a protective role towards cell death via apoptosis. This was evident within the brain cortex after exposure to short-term WBV with an increase in C/EBP homologous protein (CHOP) mRNA expression, a transcription factor known to promote apoptosis (Figure 23)(137). Prolonged ER stress can also activate other pathways resulting in upregulation of stress response genes triggering the caspase cascade leading to caspase 3 driven apoptosis (Figure 23)(203). Caspase 3 activation can also occur through extrinsic pathways involving TNFα following prolonged inflammation and has been shown to play a major role in injury-induced neuronal loss after TBI (141). Nevertheless, there was no demonstrable activation of either the ER stress or cell death pathways in the mature D21 animals (Figure 24).

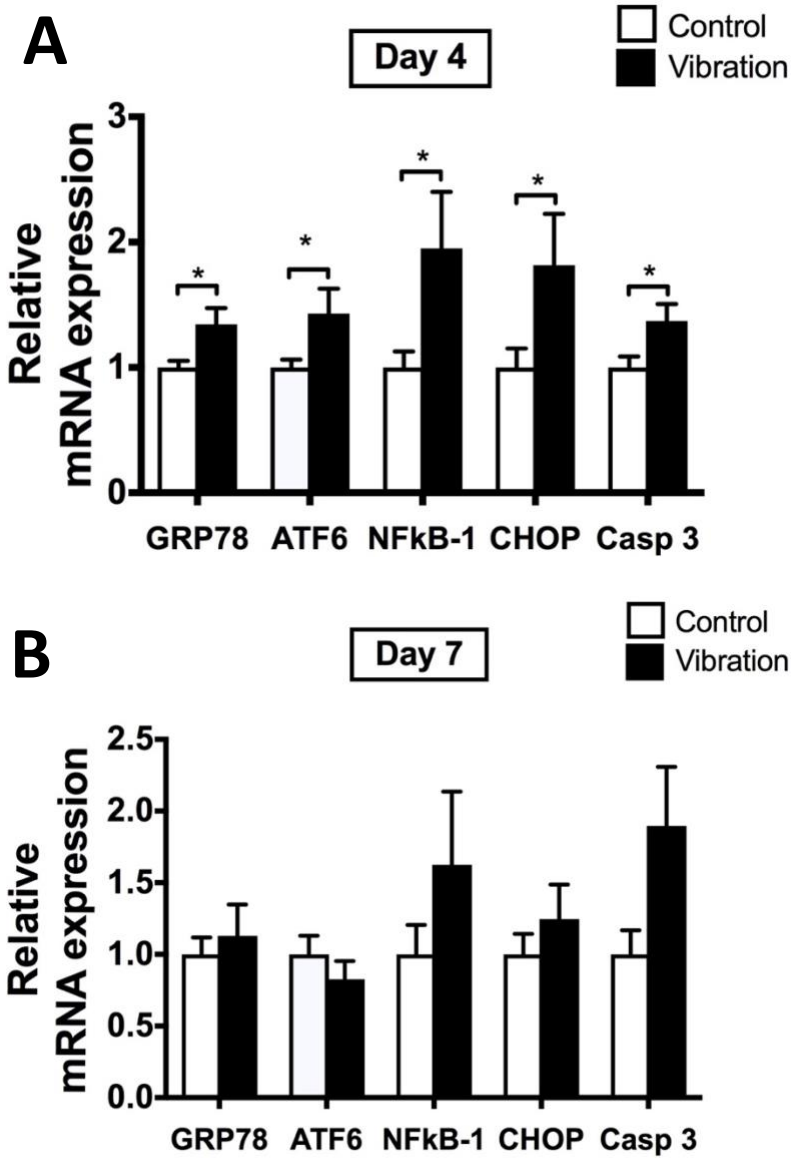


Figure 23. Relative mRNA expression of genes regulating the unfolded protein response in the brain cortex of control and whole body vibration exposed animals on (A) postnatal day 4 and (B) postnatal day 7 (n = 8/group). The genes shown are glucose regulated response (GRP78) and Activating transcription factor 6 (ATF6) and pro apoptotic genes nuclear factor kappa B subunit 1 (NFkB-1), C/EBP homologous protein (CHOP) and Caspase 3 (Casp 3). Data is shown as mean ± SEM and is expressed as relative fold change compared to control animals. *p= <0.05

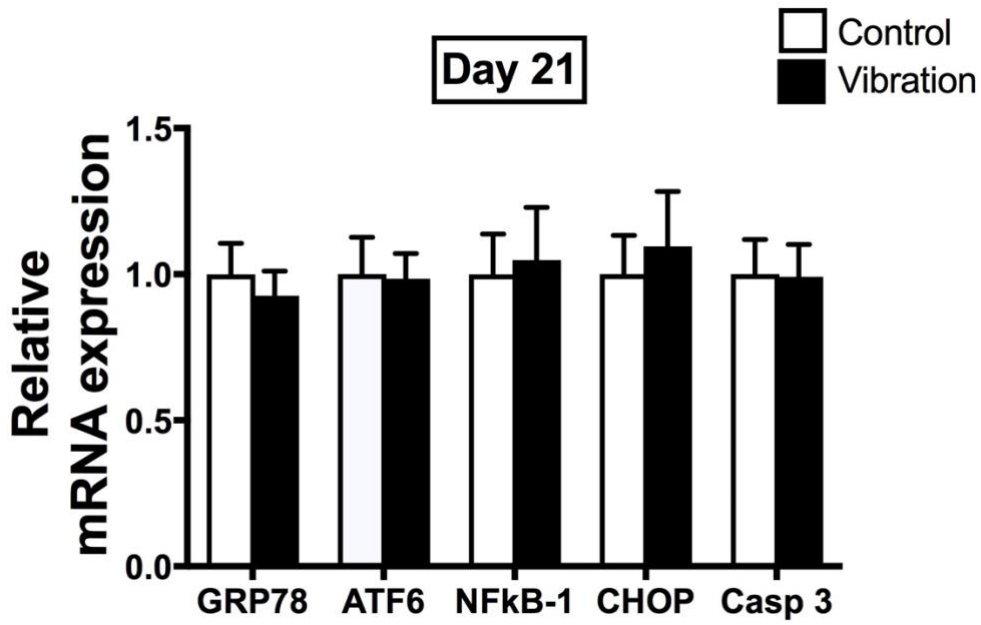


Figure 24. Relative mRNA expression of the genes regulating the unfolded protein response, glucose regulated response (GRP78) and Activating transcription factor 6 (ATF6) and pro-apoptotic genes nuclear factor kappa B subunit 1 (NFkB-1), C/EBP homologous protein (CHOP) and Caspase 3 (Casp 3) in the brain cortex of control and whole body vibration exposed animals on postnatal day 21 (n = 8/group). Data is shown as mean ± SEM and is expressed as relative fold change compared to control animals.

Short-term WBV resulted in a significant increase in TUNEL positive cells within all groups compared to control animals (Figure 25 and 26). Rink *et al* (206) demonstrated that that there was a biphasic increase in the number of apoptotic cells at 24 hours and 1 week post TBI. This was restricted to the brain cortex following controlled cortical impact brain injury in rats (142) and these findings correlate well with results following a single, short-term WBV episode.

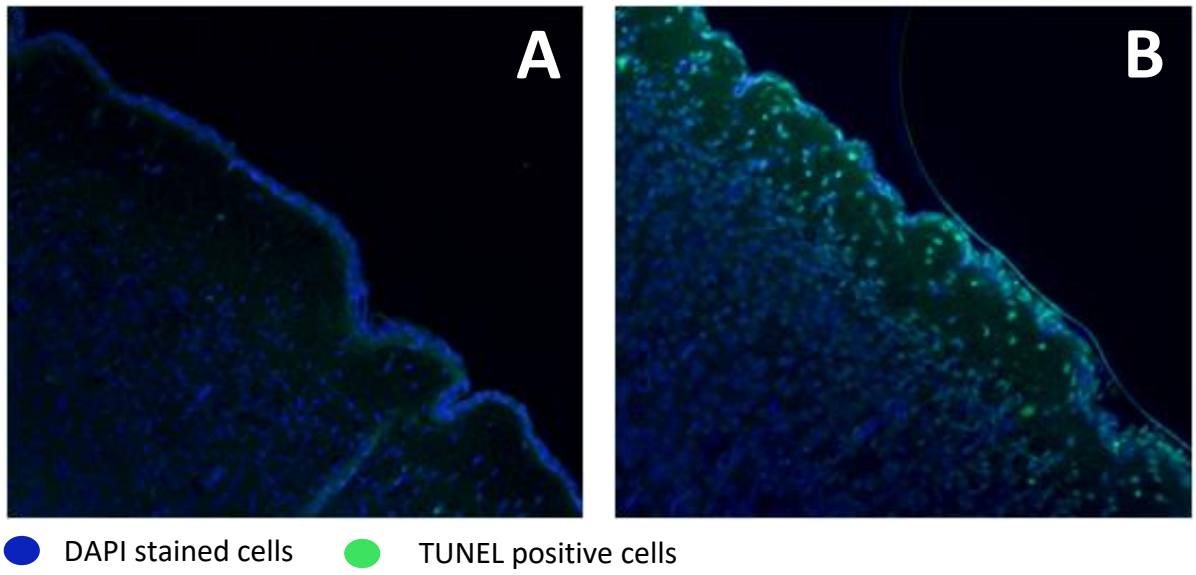


Figure 25. Representative micrographs (x 10 magnification) demonstrating 4',6-diamidino-2-phenylindole (DAPI) and terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling TUNEL staining in the brain cortex of control (A) and whole body vibration (B) exposed day 4 postnatal rodents.

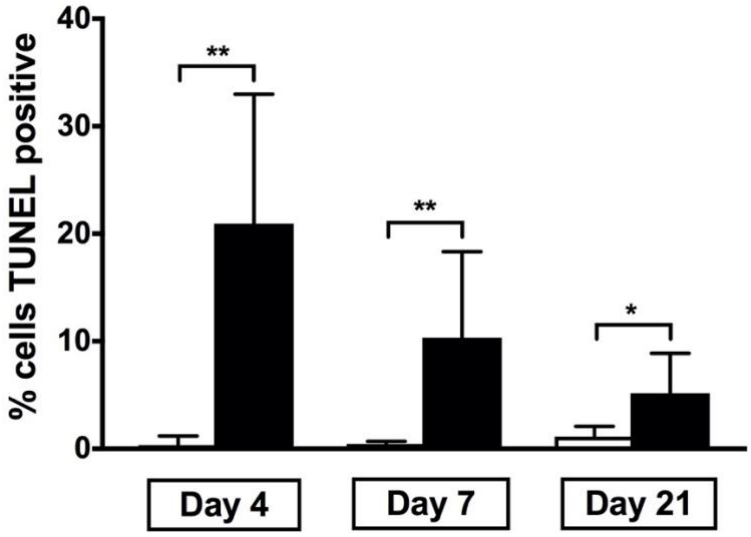


Figure 26. Total percentage of TUNEL positive cells within the designated regions of interest in the brain cortex of control and whole body vibration exposed rodents on postnatal day 4, 7 and 21 (n = 6-8/group). Data represent mean ± SD. *p = <0.05, **p = <0.01.

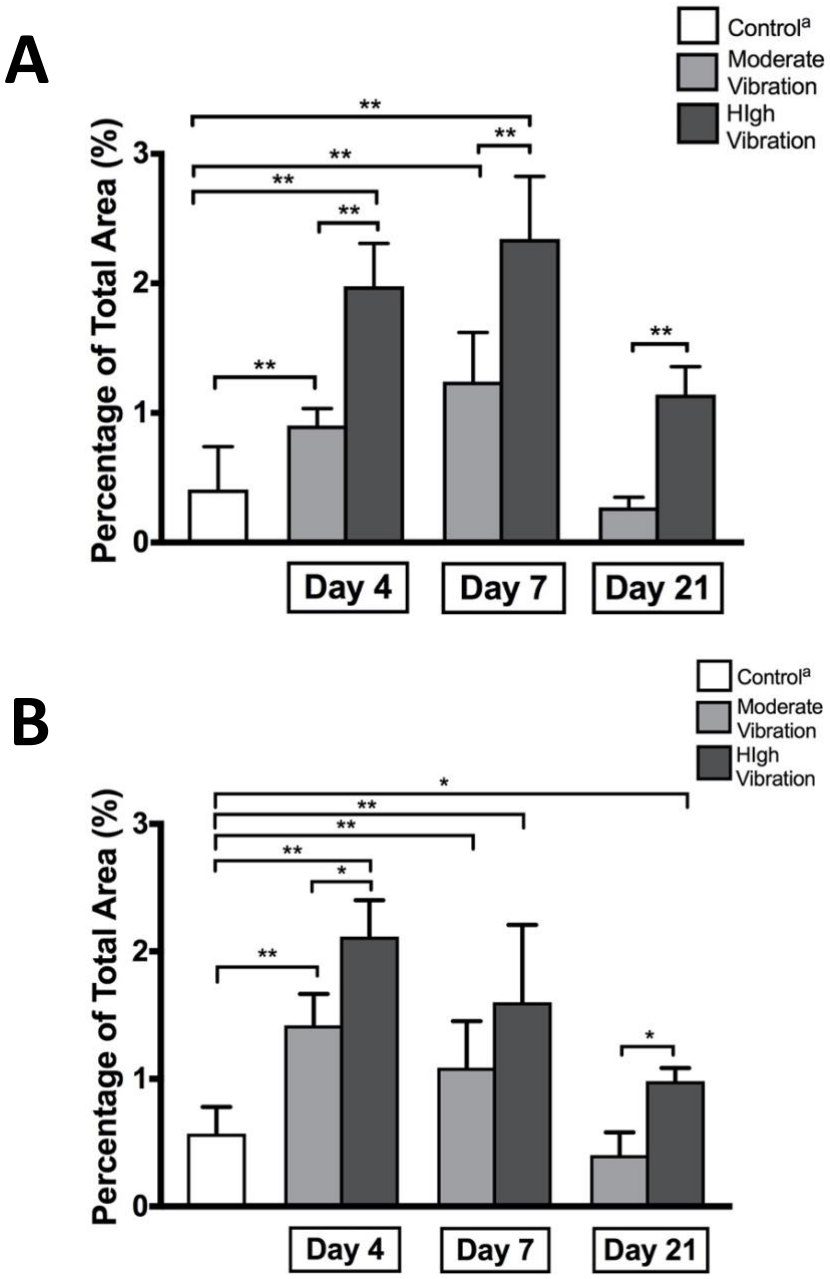
Interestingly, there was no demonstrable upregulation in mRNA expression of genes related to ER stress or cell death pathways in D7 rodents. This could reflect a lack of an inflammatory response in this age group following WBV exposure or that these pathways are activated beyond 24 hours post insult and therefore would not be detected using this single time-point. However, there was a significant increase in the proportion of TUNEL positive cells within the cortex compared to control animals, although this effect was lower in comparison to D4 animals (Figure 26). Similarly, following WBV exposure, the brain cortex in the D21 animals showed no evidence of activation of ER stress, UPR and pro-apoptotic mediators (GRP78, ATF6, NFκB-1 and CHOP) (Figure 27). Yet, evaluation of TUNEL staining

showed a significant increase in apoptotic cells post-WBV exposure compared to controls animals. Overall, the proportion of TUNEL positive cells was lower in D21 rodents compared to D4 and D7 rodents post-insult (Figure 26).

8.3.3. Dose related effects of short-term WBV

As previously described, studies that have evaluated WBV levels during neonatal transport have shown infants are exposed to levels of WBV ranging from 0.4 to 6 m/s^2 (88, 89, 100, 113), which would be deemed as little uncomfortable to extremely uncomfortable by adult standards. Hellmich et al (207) demonstrated that increasing levels of TBI (from mild to severe) are associated with dose dependent neuronal injury in adult rats 24 hours post insult. Furthermore, their analysis suggested that TBI induced increased neuroprotective gene expression at the most severe injury level. However, the effects of different WBV levels on the developing brain have not yet been determined. To gain a greater understanding of potential dose related effect of WBV on the developing brain, I explored the effects of short-term WBV at a moderate intensity on reactive microgliosis, subsequent inflammatory response, ER stress and cell death pathways.

Postnatal rat pups exposed to a moderate level of WBV, equivalent to 0.9m/s^2, had evidence of primary cell damage, such as of neuronal oedema and reactive microgliosis on histological evaluation. Although, neuronal oedema and microglial cell proliferation and migration were significantly higher in moderate intensity WBV exposed immature animals compared to controls, both measurements were significantly lower compared to animals exposed to high WBV (2 m/s^2) (Figure 27). Of note, evaluation of postnatal D21 animals exposed to moderate levels of short-term WBV did not result in a significant difference in neuronal oedema or microglial proliferation compared to controls but did demonstrate significantly lower injury than those exposed to high WBV (Figure 27).



[a] Control animals are combined to simplify display of results between WBV groups by rodent postnatal age. There was no significant difference between control groups

Figure 27. Total area of (A) neuronal oedema and (B) microglia within the designated regions of interest in the brain cortex of control, moderate (0.9m/s^2) and high (2 m/s^2) whole body vibration exposed rodents on postnatal day 4, 7 and 21 (n = 6-8/group). Data represent mean ± SD values. **p = <0.01, *p=<0.05

However, there was no corresponding increase in the pro-inflammatory cytokines TRL4, IL-1β , Il-6 or TNFα in either immature or more mature brains (Figure 28 and 29). These findings could suggest that although there was evidence of cellular damage, this was not sufficient to drive a significant inflammatory response or potentially any pro-inflammatory response had already started to resolve with predominance of anti-inflammatory cytokines and M2 type microglia within 24 hours of the sampling time. Ontological studies are required to tease out any evidence of early activation of pro-inflammatory cytokines and subsequent anti-inflammatory response. Overall, the histological findings and lack of inflammatory activation in these animals exposed to moderate WBV levels support the hypothesis that the effect of short-term WBV in the immature brain is dose related. These findings could be key in the development of safety standards for neonatal WBV exposure and would facilitate evaluation of strategies to minimise WBV exposure through targeting exposure levels known to result in minimal insult.

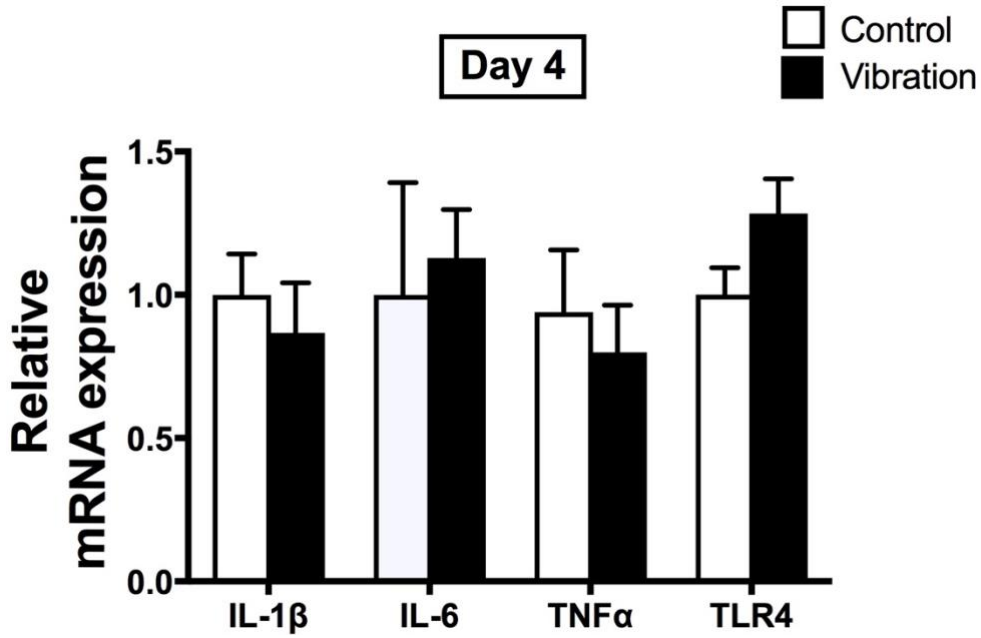


Figure 28. Relative mRNA expression of the pro-inflammatory genes Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), Tumour Necrosis Factor alpha (TNFα) and Toll-like receptor 4 (TLR4) in the brain cortex of control and moderate whole body vibration (0.9m/s^2) exposed postnatal day 4 rodents (n = 5-6/group). Data is shown as mean ± SEM and is expressed as relative fold change compared to control animals.

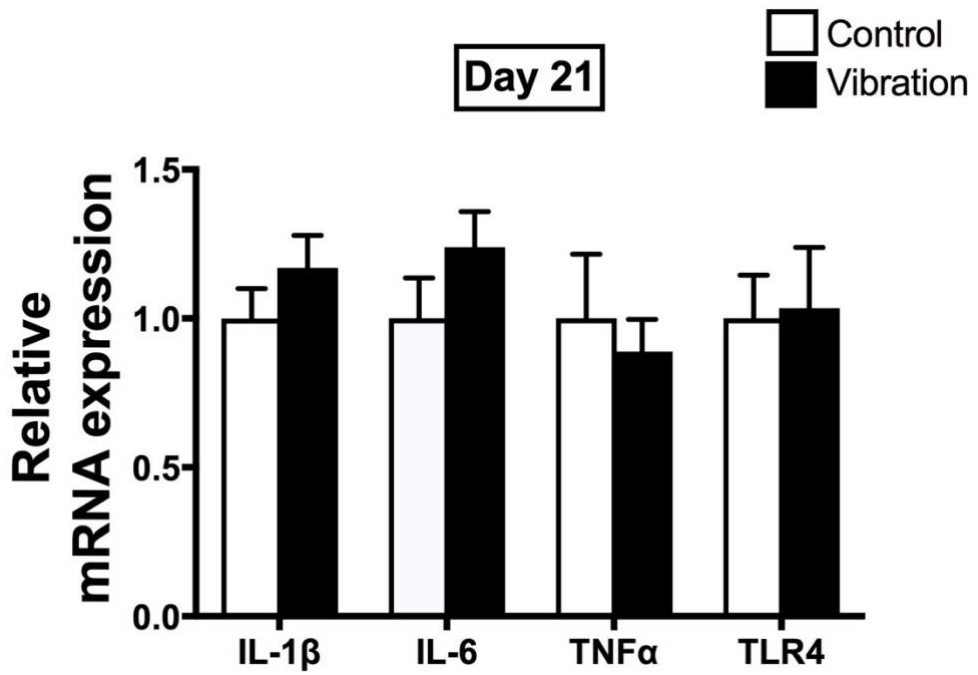


Figure 29. Relative mRNA expression of the pro-inflammatory genes Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), Tumour Necrosis Factor alpha (TNFα) and Toll-like receptor 4 (TLR4) in the brain cortex of control and moderate whole body vibration (0.9m/s^2) exposed postnatal day 21 rodents (n = 6/group). Data is shown as mean ± SEM and is expressed as relative fold change compared to control animals.

Similarly, assessment of both D4 and D21 exposed to lower levels of short-term WBV did not result in activation of ER stress or cell death pathways (Figure 30 and 31).

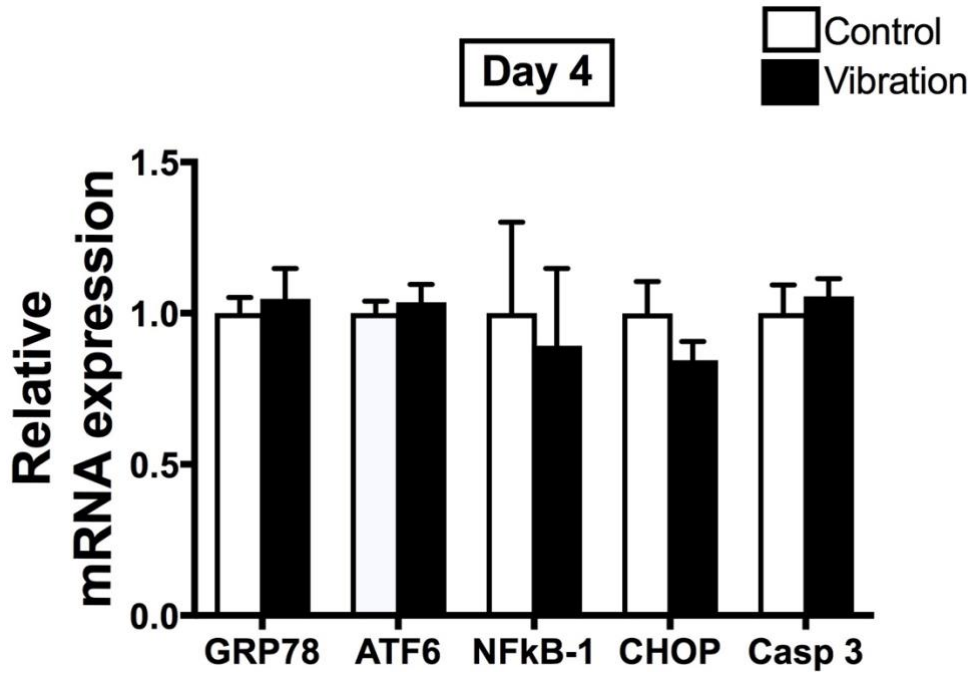


Figure 30. Relative mRNA expression of the genes regulating the unfolded protein response, glucose regulated response (GRP78) and Activating transcription factor 6 (ATF6) and pro apoptotic genes nuclear factor kappa B subunit 1 (NFkB-1), C/EBP homologous protein (CHOP) and Caspase 3 (Casp 3) in the brain cortex of control and moderate whole body vibration (0.9 m/s^2) exposed animals on postnatal day 4 (n = 5-6/group). Data is shown as mean ± SEM and is expressed as relative fold change compared to control animals.

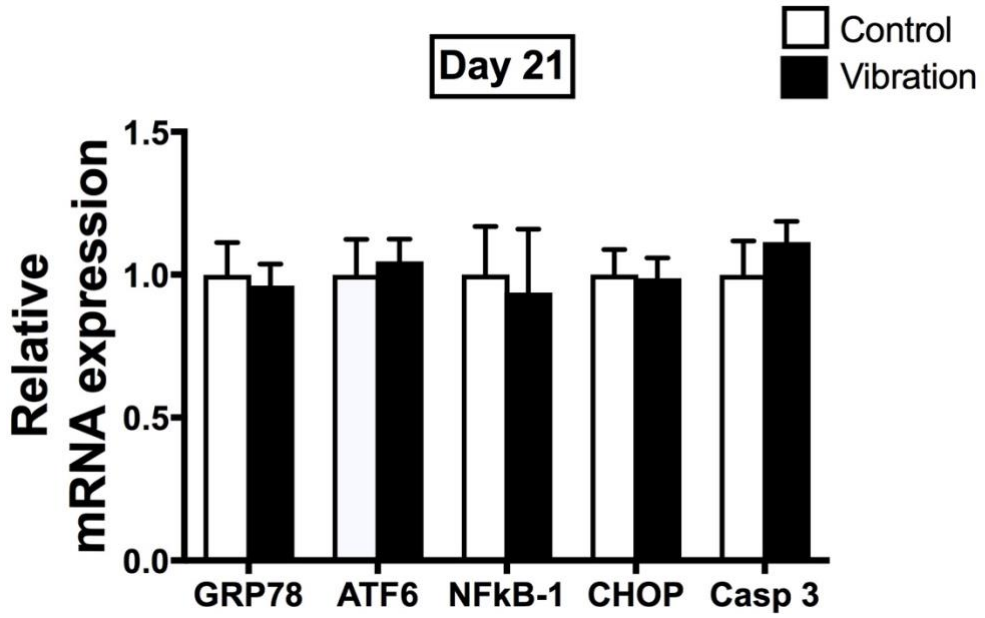


Figure 31. Relative mRNA expression of the genes regulating the unfolded protein response, glucose regulated response (GRP78) and Activating transcription factor 6 (ATF6) and pro apoptotic genes nuclear factor kappa B subunit 1 (NFkB-1), C/EBP homologous protein (CHOP) and Caspase 3 (Casp 3) in the brain cortex of control and moderate whole body vibration (0.9 m/s^2) exposed postnatal day 21 rodents (n = 6/group). Data is shown as mean ± SEM and is expressed as relative fold change compared to control animals.

8.3.4. Corticosterone

Immature rodents (D4 to D14), like human infants, exhibit a stress hyporesponsive period (208). This is reflected in my findings, that there was a lack of corticosterone release in the immature (D7) rodents in either the control or treatment cohort (Figure 32). The release of glucocorticoid steroids in response to neurological insults act to suppress inflammation through suppression of NFkB-1 activation, reducing microglial ROS production and decreasing expression of inflammatory cytokines (IL-1β and TNFα)(209). Therefore,

inadequate glucocorticoid production secondary to the stress hyporesponsive period can result an increased inflammatory response due to lack of inhibition of cytokines that normally counter-acted by glucocorticoid. Interestingly, postnatal D21 animals showed a significant increase in corticosteroid release in response to short-term WBV exposure (Figure 32). Conversely, excessive glucocorticoid release can have a detrimental affect resulting in increased expression of pro inflammatory cytokines (209). Furthermore, excessive stress response and subsequent glucocorticoid exposure could potentiate the excitotoxic effect of concurrent neurological insults through increased glucocorticoid dependent glutamate release (210). This response could contribute to the significant increased risk of seizures seen in transported infants with HIE born in a non-cooling centre (Paper 5).

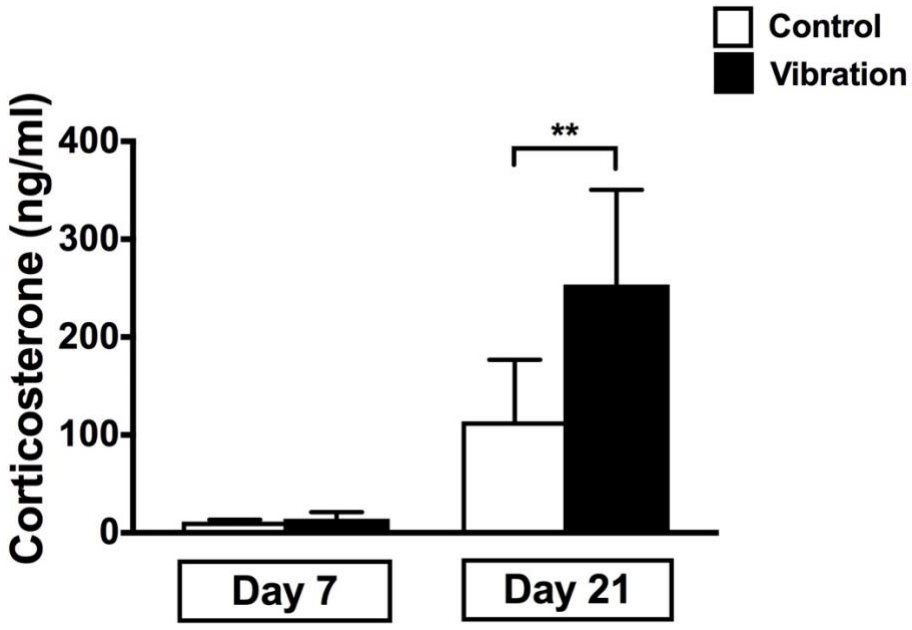


Figure 32. Corticosterone concentration in the serum of postnatal day 7 and 21 rat pups (n = 6-8/group) 24 hours after short-term whole body vibration exposure (vibration group only). Data is presented as Mean ± SD. ** p = <0.01

8.3.5. Potential biomarkers of brain injury

S100B and GFAP have been used as biomarkers of brain injury following traumatic insult and correlate well with neurological outcomes (121, 153, 154, 156). As previously demonstrated, short-term WBV results in both neuroinflammation and cell apoptosis in the brain cortex. It is therefore conceivable these biomarkers could also be used in infants who are exposed to WBV following PNT to monitor brain injury and identify infants at high risk of adverse outcomes. I have explored GFAP gene expression and S100B serum concentration after WBV insult to allow a greater understanding of the association of short-term WBV and biomarkers of perinatal brain injury.

The severity of neuroinflammation and cell apoptosis within the brain cortex secondary to short-term WBV exposure is age dependent. This was greatest in postnatal D4 animals and demonstrated a decline with increasing age. As anticipated, examination of GFAP gene expression was upregulated in postnatal D4 rodents after WBV insult, however, there was no difference in more mature animals (Figure 33). Similarly, S100B concentration was only increased in the serum of immature D4 rodents (Figure 34) and not at D21. These findings suggest these biomarkers could be used to monitor brain injury in extremely preterm infants. However, currently there is no evidence evaluating serum concentrations of either GFAP or S100B in high-risk infants post PNT. This could potentially be used to guide research aimed at reducing brain injury. The correlation of these biomarkers with neurological outcomes post-TBI raises the possibility for their use following WBV exposure in transported infants to aid prognostic evaluation. Careful evaluation and interpretation of their use would be required, as biomarker release secondary to early IVH prior to WBV exposure could potentially mask more subtle changes in concentration levels.

Many infants with HIE need early PNT to a centre providing TH (21). There is evidence to suggest that the transportation process could contribute to increased adverse neurological outcomes in infants with HIE (Paper 5). Both GFAP and S100B are significantly elevated in infants following hypoxic insult and these correspond with HIE severity (121, 156). It has been suggested that there could be a synergistic pathway between hypoxia and inflammation, which may exacerbate brain injury (211). Despite my findings of a lack of biomarker response following short-term WBV in D21 rodents, further studies are warranted to evaluate the potential of these biomarkers to detect any additional secondary brain injury following a hypoxic insult.

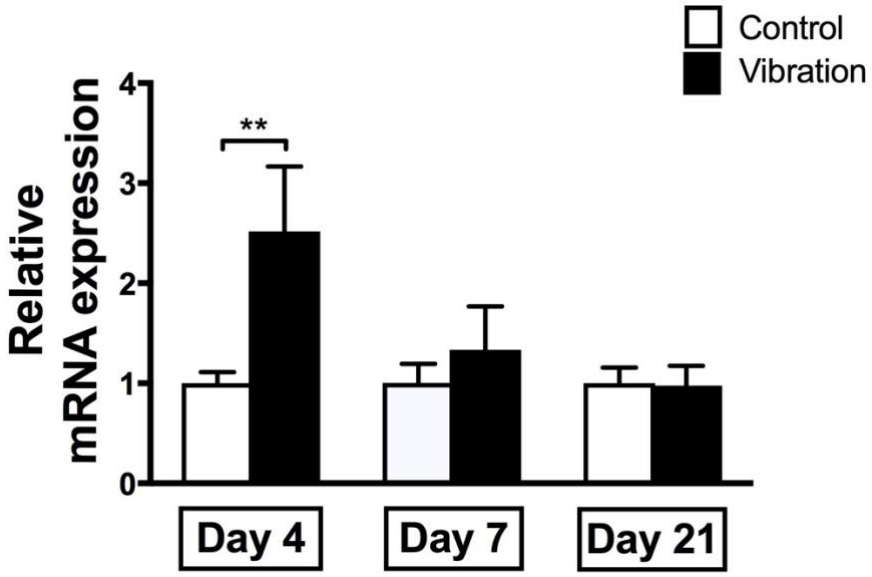


Figure 33. mRNA abundance of Glial Fibrillary Acidic Protein (GFAP) in the brain cortex of postnatal day 4, 7 and 21 rats (n= 7-8/group). Data presented as mean ± SEM.

**** p = <0.01**

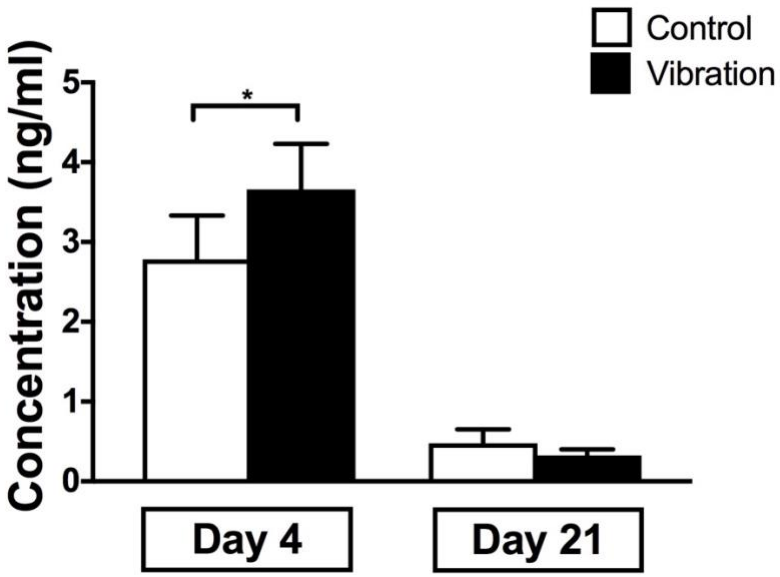


Figure 34: S100B concentration in the serum of postnatal day 4 and 21 rats (n= 6-8/group) 24 hours after short-term whole body vibration exposure (vibration group only). Data presented as mean ± SD. * p = <0.05

8.3.6. Overview of chapter results

Table 4 provides a summary overview of all my presented findings per rodent age group.

	Moderate WBV (0.9 m/s²)			**High WBV (2 m/s²)**		
	Day 4 (n = 11)	**Day 7 (n= 13)**	**Day 21 (n= 12)**	**Day 4 (n = 14)**	**Day 7 (n= 16)**	**Day 21 (n= 16)**
Neuronal oedema	↑↑	↑↑	↔	↑↑	↑↑	↔
Reactive microglia	↑↑	↑↑	↔	↑↑	↑↑	↑
IL-1ß	↔		↔	↔	↔	↔
IL-6	↔		↔	↑↑	↔	↔
TNFα	↔		↔	↑↑	↑	↔
TLR4	↔		↔	↑↑	↑	↔
Corticosterone					↔	↑↑
GRP78	↔		↔	↑	↔	↔
ATF6	↔		↔	↑	↔	↔
NFκB-1	↔		↔	↑	↔	↔
CHOP	↔		↔	↑	↔	↔
Caspase 3	↔		↔	↑	↔	↔
TUNEL +ve cells				↑↑	↑	↑
GFAP				↑	↔	↔
S100B				↑↑		↔

WBV, Whole body vibration; m/s², metre/second²; IL-1β, Interleukin 1 beta; IL-6, Interleukin 6; TNFα, Tumour Necrosis Factor alpha; TLR4, Toll-like receptor 4; **,** GRP78, glucose regulated response; ATF6, Activating transcription factor 6; NFkB-1, nuclear factor kappa B subunit 1; CHOP, C/EBP homologous protein; GFAP, Glial Fibrillary Acidic Protein. Data are compared to control animals.
↑↑p = <0.01, ↑ p = <0.05, ↔ represents data were measured but not significant

Table 4. Overview summary of findings from rodents exposed to whole body vibration per age group.

8.3.7. Discussion

In summary, the data presented from these experiments support the theory that WBV induced injury results in primary direct cellular damage similar to that associated with TBI. These findings suggest the severity of injury is age dependent and demonstrated a decline with increasing age. Exposure to short-term WBV equivalent to a typical ambulance journey can trigger both cellular inflammation and apoptosis within the immature brain cortex. This cell injury is more extensive in the extremely immature brain. These findings further support epidemiological studies that have associated PNT with adverse neurological outcomes in extremely preterm infants (11, 14, 23, 52) and therefore, the need to avoid unnecessary PNT. However, this is not always possible so additional work to reduce the level of WBV transmitted to the infant from the environment (e.g. modification of the mattress medium) is required to minimise potential neurological insult. Although short-term WBV exposure resulted in significant cellular apoptosis, it will be important to assess the effects on long-term neurological function and outcomes.

Of importance, the lungs of these animals have also been examined with both histology and the same gene expression pathways (work undertaken by fellow PhD student). The lungs of the rodents at 4 and 7 days have no evidence of injury or inflammation compared to the controls. This would suggest the developing brain is more sensitive to this type of noxious stimulus.

Although the impact of short-term WBV is reduced in the more mature brain, this could still have important implications. Term infants who undergo early PNT are most likely to have an underlying diagnosis of HIE, with resulting onset of cell damage, inflammation and apoptosis. There is evidence to suggest there is a synergistic pathway between hypoxia and

inflammation, which can exacerbate brain injury (211). Seizure activity in infants with HIE occurs due to secondary brain injury post hypoxic insult (212). My finding that, for infants with moderate/severe HIE, birth in a centre without immediate access to active TH is associated with increased seizure burden could provide supporting evidence that expose to WBV during PNT exacerbates brain injury, perhaps in tandem with suboptimal cooling. Gaining a greater understanding of the potential for short-term WBV to heighten cell injury following a hypoxic insult is essential to avoid worsening long-term neurodevelopmental outcomes.

The underlying reason for a greater degree of injury as a result of short-term WBV exposure in the more immature animals is not clear. However, it could be as a consequence of:

- the physical structure of the developing brain. The degree of myelination and water content of the brain tissues varies with age (213). This could potentially result in differences in the degree of transmission of WBV through the tissues, with forces being more easily transmitted throughout the immature brain (135).
- an imbalance between excitatory and inhibitory pathways of neuroinflammation in the developing brain, with the more immature brain displaying heightened sensitivity to excitotoxicity after a traumatic insult. The reduced anti-oxidant capacity in the more immature brain leads to accumulation of ROS that exacerbates cell damage, promotes microglial activation and further inflammation (214, 215).
-delayed maturational expression of genes involved in the suppression of apoptosis (Bcl-2 and p35) (216).
- increased neurogenesis and neural migration with decreasing maturity. Immature pre-oligodendrocytes have increased susceptibility to oxidative stress, which can result in

exacerbated neuronal death and dysfunctional growth in neuronal processes following brain injury (211).

- increased vulnerability of neurons and glial cells to apoptosis during a certain period of maturation and differentiation process. The preterm brain has a greater degree of physiological cell apoptosis than term infants as a result of high levels of neuroplasticity in this age group (135).

- the stress hyporesponsive period exhibited by immature rodents (208) and human preterm infants. As previously detailed, reduced glucocorticosteroid release during this period in the more immature animals could result in a lack of suppression of the inflammatory pathway.

8.3.8. Limitations of the rodent model

The results presented in this chapter have used a novel neonatal rodent model to evaluate the effect of short-term WBV on the developing brain. However, there are limitations with the use of animal models and subsequent translation into human outcomes.

Potential limitations of my novel rodent model are:

- the position of the rodent within the holding box. Human infants are transported supine with the head directly placed onto a mattress, whereas the rat pups are in a standing position. Therefore, the transmission of vibration through the body of the rat pups could be attenuated by the muscle tone within the paws, torso and neck.

- the lack of restraints. Safety harnesses are routinely used during neonatal transportation, which aim to minimise movement of the infant within the transport incubator. As detailed in Appendix 1, I was unable to use restraints to immobilise the rat pups during WBV

exposure. However, movement within the holding box was minimised by the small size of the chamber in conjunction with a nest of bedding. This could, however, potentially result in additional movement of the rat pup.

- the use of simulated ambulance journeys. Infants undergoing transportation are exposed to varying levels of WBV that can be affected by bumps in the road, stopping, braking and accelerating throughout the journey. Although, brief jolts and pauses were included within the vibration programme design, Dr. Bloor was unable to incorporate acceleration and deceleration movements.

- increased water to white matter ratio within the rodent brain. This could potentially impact on the transmission of vibration waves through the brain tissue.

- differences in relative body mass and composition between rodents and human infants. Human infants have a higher body mass and are likely to experience less body movement as a result of WBV exposure (217). Additionally, greater proportion of body fat in humans can as a dampening material through absorption of vibrational energy (218).

- euthanasia at 24 hours post exposure. Therefore, limiting evaluation of temporal changes within the inflammatory and apoptotic pathways.

Overall, my findings using this novel rodent model have demonstrated that short-term WBV vibration could contribute to brain injury. However, future work using a larger animal model would allow mitigation of some limitations associated with this rodent model. For example, piglets have a closer correlation to human brain anatomy, similar weight to human infants and could be restrained in a supine position. Moreover, it is important extended studies to evaluate evolution of the resulting brain injury beyond 24 hours post insult and long term functional and cognitive neurological outcomes.

Chapter 9. Conclusions

The overall aim of this thesis was to evaluate the impact of a centralised approach to neonatal care on outcomes for both infants and parents. My findings provide up to date evidence that the rates of IUT are decreasing, whereas conversely early PNT of extremely preterm infants is increasing. However, early PNT of these infants is associated with an increased risk of severe IVH compared to inborns, which can result in severe long-term neurodisability.

The centralised care pathway ultimately results in the management of high-risk infants in a non-BH, which is often a long distance from parent's home. It is important to acknowledge the additional physical and financial burden this could place on a family. Recognition of the potential source of stressors can allow for support services to be developed and highlights the need for essential resources, such as adequate provision of accommodation and mental health support.

HIE is the leading cause of brain injury in the UK for term infants, though an increasing number of infants are being managed with TH outside of evidence-based guidance. A large number of infants are born in non-cooling centres without immediate access to active TH. Birth in a non-cooling centre is associated with worse short-term neurological outcomes, but it remains to be ascertained whether this persists into later life. This finding could potentially be due to suboptimal TH but also secondary to exposure to adverse stimuli, such as WBV, during the transport process.

The novel findings presented in this thesis confirms that short-term WBV exposure can lead to inflammation and activation of apoptotic pathways in the cortex of the developing brain similar to that associated with TBI. My findings that the severity of insult is age dependent, as demonstrated by a decline with increasing age, supports the hypothesis that the more immature brain appears to be more at risk of injury than the mature brain. This highlights the vulnerability of extreme preterm infants to potential injury, the need to advocate IUT in threatened preterm labour and for careful consideration when relocating an infant due to cot capacity issues.

There is evidence demonstrating neonatal patients are exposed to excessive levels of WBV during transportation. However, it is unclear whether the brain injury associated with WBV impacts on long-term neurological outcomes. Additionally, the potential of WBV to exacerbate secondary brain injury following already established haemorrhagic or hypoxic-ischaemic insult is yet to be ascertained. Understanding the potential mechanism of causation could allow strategies to be developed aimed at reducing long-term neurological deficits. I was able to demonstrate a dose-related association between WBV exposure and the activation of inflammatory and apoptotic pathways. Monitoring WBV levels and development of methods to minimise transmission of harmful vibration to an infant during the transport process is essential to reduce associated adverse effects. Furthermore, there is growing interest in new pharmacological neuroprotective agents aimed at reducing secondary cell damage and subsequent development of long-term adverse outcomes through targeting the inflammatory or apoptotic pathways. However, the findings presented in this thesis suggest we need to focus on strategies to reduce PNT of these high-risk infants, where possible, to prevent potential harm.

Chapter 10. Future work

The findings in my thesis demonstrate that centralisation of neonatal intensive care can lead to adverse neurological outcomes in high-risk infants. Future work should focus on strategies to increase the proportion of IUTs in the UK. This maybe through development of guidelines to recognise women who could undergo IUT earlier and logistical planning of service provision, such as ensuring staff are available to accompany women at short notice. Further evaluation of the potential benefits of a centralised referral system alongside up to date maternity bed and cot status to facilitate the co-ordination of this care pathway is required. These interventions could reduce unnecessary early PNTs and the risk of associated adverse outcomes. In particular strategies, such as provision of extra beds in level 3 centres (159), to address factors contributing to lack of cot capacity can also reduce this burden.

Additional stress placed on a family whilst their infant is being cared for in a specialist centre could increase the risk of poor mental health and have a considerable financial impact. In light of my findings, along with recent reports from Bliss (46, 57, 58), service providers should focus on improving the care we deliver to these families and address current deficits in financial support, accommodation and psychological support services.

The data presented within my thesis are the first to describe how the transportation process and subsequent WBV exposure experienced during the journey could contribute to brain injury through onset of neuroinflammation and apoptosis. However, investigation of both the inflammatory and apoptotic pathways beyond 24 hours post insult to evaluate evolution

of the resulting brain injury and potentially determine key therapeutic windows for neuroprotective agents. Therapies such as erythropoietin and melatonin have shown potential (197). Extended studies could examine the potential of these therapies to reduce brain injury prior to translation into human studies. It is essential using a suitable model, to evaluate the long-term functional and cognitive neurological outcomes following WBV insult to determine whether the associated brain injury seen translates into neurological deficit. Rodent models have been invaluable in the development of neurological targeted drugs, although the translation to human species is not perfect. Rats mature at an accelerated rate compared to humans and have a much shorter gestational period (21 days vs 270 days) (219). Additionally, there is a paucity of white matter, an area which is particularly vulnerable to injury in the preterm infant (211). For evaluation of WBV exposure the physical weight, composition and position of the rodent's head in relation to the vibration platform in my study could potentially impact on the transmission of vibration waves through the brain tissue. Therefore, it is essential for additional studies to explore the effects of short-term WBV on the brain either using larger animal model, which have a closer correlation to human brain anatomy or through evaluation of biomarkers of brain injury in transported human infants.

There is growing interest in the use of biomarkers present in bodily fluids to allow detection of brain injury (121, 153, 154). These biomarkers have proven to correlate well with prognostic outcomes, even in the absence of detectable injury using imaging modalities (121, 155). I have demonstrated that exposure to WBV at levels equivalent to an average neonatal ambulance journey resulted in detection of S100B within the serum and upregulation of GFAP. Further work is required to determine whether upregulation of GFAP

translates into a detectable protein, which in turn can be detected in the serum following WBV insult. However, it could be feasible for future human neonatal studies to utilise these markers, which are easily detected in the serum to 1) identify transport associated brain injury 2) evaluate correlation with prognosis 3) monitor strategies aimed at minimising exposure to excessive vibration. It is possible that by implementing strategies to minimise the transmission of WBV to an infant (e.g. through modification of the ambulance, mattress materials and harness) we could help reduce the risk of adverse long-term neurological outcomes in high-risk infants. I have been involved in a further study during my PhD period to evaluate the feasibility of using these biomarker as end points in studies. This study aimed in the initial phase to quantify both noise and WBV exposure levels experienced during neonatal transportation and correlate these with both physiological and biochemical changes (including S100B) that may occur. The results from this phase will be used to develop and evaluate strategies aimed at reducing WBV transmission to infants. Appendix 2 provides an overview of a study protocol that I help design and gain ethical approval demonstrating on-going work in this area of interest.

Finally, there is evidence to suggest earlier cooling is associated with improved outcomes (75, 76). Infants who are born outside of a cooling centre have a greater risk of not achieving optimal therapeutic temperature within the key treatment window. It would be intriguing to explore whether ensuring an infant had a temperature within the therapeutic range prior to transportation could provide better neuroprotection to these infants and potentially reduce associated adverse outcomes seen in infants born in non-cooling centre. Future work should focus on targeting barriers (e.g. service provision, staff education and improved recognition) that prevent these infants achieving effective cooling. Active TH is a

safe and effective treatment for moderate/severe HIE (187, 220). Provision of active TH at all birthing centres prior to transportation with support from level 3 centres could mitigate the risk of delayed cooling whilst awaiting transfer. To allow development of a co-ordinated service the costs of set up (e.g. equipment, staff training) versus potential benefits of reduction in long-term neurodisability needs to be carefully considered.

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List of Publications and Presentations

Peer- reviewed publications

- **Shipley L**, Gyorkos T, Dorling J, Tata L, Szatkowski K and Sharkey D. Risk of Severe Intraventricular Haemorrhage in the First Week of Life in Preterm Infants Transported Before 72 hours of age. Pediatr Crit Care Med. 2019;20(7):638-44
- Gupta N, **Shipley L**, Goel N, Browning-Carmo K, Leslie A, Sharkey D. Neurocritical care of high-risk infants during inter-hospital transport. Acta Paediatrica. 2019; 108: 1965-1971.

Peer-review publications (Co-author during PhD period)

- Pickup L, Lang A, **Shipley L**, Henry C, Carpenter J, McCartney D, Butler M, Hayes-Gill B, Sharkey D. Development of a clinical interface for a novel newborn resuscitation device: Human factors approach to understanding cognitive user requirements. JMIR Human Factors. 2019; 6(2):e12055.
- Henry C, **Shipley L**, Ward C, Mirahmadi S, Liu C, Morgan S, Crowe J, Carpenter J, Hayes-Gill B, Sharkey D. Accurate neonatal heart rate monitoring using a new wireless, cap mounted device. Acta Paediatrica. 2020; 00:1-7.

Conference presentations (Oral)

- Gyorkos T, **Shipley L**, Szatkowski L, Sharkey D. The Effect of antenatal steroid course on Intraventricular Haemorrhage in Preterm Infants transported in Early life, 2016. Transport of High Risk Neonates, Copenhagen.
- **Shipley L**, Bloor I, Sharkey D. The effects of short term whole body vibration, as experienced during neonatal ambulance transportation, on the early developing rodent brain, 2017. Transport of High Risk Neonates, Oxford.
- **Shipley L**, Bloor I, Sharkey D. A comparison of the effects of short term whole body vibration, as experienced during neonatal ambulance transportation, on the developing brain. 2017. Joint European Neonatal Society, Venice.
- **Shipley L**, Bloor I, Sharkey D. Whole body vibration, as experienced during neonatal ambulance transportation, as a mechanism of injury in the developing brain: a new rodent model, 2018. Neonatal Society, London.
- **Shipley L**, Bloor I, Sharkey D. Effects of short term whole body vibration, as experienced during neonatal ambulance transportation, on the developing brain: Development of a relevant animal model, 2018. Paediatric Academic Society, Toronto.
- **Shipley L**, Szatkowski L, Sharkey D. The hidden impact of centralised neonatal intensive care on the family: A UK population study, 2018. UK National Neonatal Transport Group Conference, Liverpool.
- **Shipley L**, Tata L, Sharkey D. Increasing use of therapeutic hypothermia for HIE outside of the current evidence-base: A national population study 2011-2016. 2019. Joint European Neonatal Society, Maastricht.

Conference presentations (Poster)

- Yeo M, Henry C, Hill S, **Shipley L**, Blaxter L, Hayes-Gill B, Crowe J, McNally D, Leslie A, Sharkey D. Exposure of preterm head to excessive shock and vibration during inter-hospital transport, 2015. UK National Neonatal Transport Group, Brighton.
- Gyorkos T, **Shipley L**, Szatskowski L, Sharkey D. Does early inter-hospital transport of preterm infants increase the risk of intraventricular haemorrhage? 2016. UK National Neonatal Transport Group, Bristol.
- Gyorkos T, **Shipley L**, Szatkowski L, Sharkey D. Early Interhospital Transport of Preterm Infants is associated with severe IVH irrespective of antenatal steroid course, 2016. European Academy of Paediatric Societies, Geneva.
- **Shipley L**, Forster C, Burn S, Sharkey D. Uncut versus pre-cut Endotracheal tube strategies for intubation at birth, 2016. European Academy of Paediatric Societies, Geneva.
- **Shipley L**, Szatkowski L, Sharkey D. The changing pattern of In-utero and postnatal transfer of infants born <28 weeks' gestation in the UK from 2011-2016. UK National Neonatal Transport Group, Liverpool.

Appendix 1

Supplementary methods – The effect of whole body vibration on the developing brain

1.1. Subjects

Eight Sprague Dawley rats with timed pregnancy to ensure accuracy of date of delivery were obtained from Charles River (Kent, UK). Rat pups of D4 (n = 25), D7 (n = 29) and D21 (n = 28) postnatal ages were used as cohorts. The rats were sexed, numbered and randomly assigned to each of the timed cohorts then either the control or intervention group (http://www.graphpad.com/quickcalcs/randomize1.cfm). For each time cohort female rats were exposed to a higher measure vibration programme. All animals were housed with their mothers and had free access to maternal milk and water. The environmental temperature was kept constant at 21^{o}C and with regulated light/dark exposure to minimise stress exposure. Given the novelty of the study, D7 and D21 cohorts were evaluated in the first instance to ensure the animals were not overly stressed prior to D4 cohorts. This alongside the inability to predict the quantity of each gender per cohort has led to small differences in the number of animals in each cohort. The study was approved by the University of Nottingham Animal Welfare and Ethical Review Board and were carried out in accordance with the UK Animals (Scientific Procedures) Act of 1986 (project license: PPL 40/3560).

1.2. Vibration exposure

Intervention rats were placed, un-anaesthetised, into a segmented box fixed to a vibration platform (Multi-function 3D rotator PS-M3D, Grant-bio, UK) and control rats were placed in a container within the same room for the duration of the vibration programme. Both vibration compartments and control holding boxes contained a small amount of bedding material to reduce stress due to maternal separation and minimise movement within the

box. The accelerometers (Triaxial, model 3093B, Dytran, Doncaster, UK) were fixed to either side of the vibration box in marked positions to ensure accurate relocation for subsequent cohorts. WBV was measured using a vibration level meter (model SV106, Svantek, Poland). Two vibration programmes were devised by Dr Ian Bloor, using ambulance vibration data from Blaxter *et al* (88) and Shenai *et al* (113) to reflect the range of WBV exposure levels reported within these studies. Both studies measured WBV levels via placement of an accelerometer on the neonatal head during transportation, allowing more accurate estimation of exposure.

For each time cohort, the animals were separated by gender. The male rodents were exposed to an 86 minute programme at an average 0.9 m/s^2 (moderate) intensity. This reference level represents both the average WBV experienced by the neonatal head and length of time for transportation, as demonstrated by Blaxter *et al* (88) (unpublished data used for average journey length was obtained directly from the study authors). Female rodents were exposed to a higher intensity 90 minute programme at an average of $2m/s^2$ based on exposure levels reported by Shenai *et al* (113). Both programmes included brief pauses and jolts ($2m/s^2$ (moderate programme) and $4\ m/s^2$ (high programme)) to mimic a vehicle stopping and road defects or speed bumps. During testing the vibration box was weighted with masses equivalent to rodent pups. The male programme was slightly shorter than the female programme due to the pre-set length of motion cycles used on the vibration platform and need to have an overall lower average WBV exposure. Although, Shenai *et al* (113) reported higher levels of WBV during transportation, we were unable to expose the neonatal rat pups to sustained levels $>2m/s^2$, which could be deemed as extremely uncomfortable (108). Furthermore, we were unable to restrain the animals within the holding box. This was due to the use of neonatal rodents and guidance as per

Animal (Scientific Procedures) Act 1986 (Section 2: Principles of replacement, reduction and refinement).

At the end of the allocated programme both the intervention and control rats were placed back with their mothers for 24 hours prior to euthanasia by cervical dislocation and death confirmed via decapitation as per Schedule 1 Animal (Scientific Procedures) Act 1986. Tissues were collected and either placed into formaldehyde or snap frozen in liquid nitrogen and stored at -80 for subsequent genetic and histopathological analysis.

1.3. Histopathology analysis

Brain tissue was kept in formaldehyde for 5 days prior to embedding in paraffin wax. Cross sections of the brain tissue were obtained using a microtome at 5μm in the coronal plane and mounted onto Superfrost slides. Three sections were collected for each animal. One set of slides were stained using Periodic Acid Schiff staining (Thermo Scientific, Loughborough, UK) following manufacturers' instructions. The sections were examined by light microscopy, using a x20 objective lens. Blinded evaluation of histology slides was undertaken using ImagePro software (Media Cybernetics, USA). Six non-overlapping regions of interest (ROI) in the frontal cortex, to include all six anatomical layers, were identified per animal. The area of interstitial space surrounding cells, indicating oedema, and glial cells was calculated per ROI. These values were averaged to obtain a single value for both area of white and glial cells per animal.

A further set of slides from female D4, 7 and 21 rats were used for terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) analysis using Promega TUNEL system kit following manufacturer's instructions. All slides were stained with 4',6-diamidino-2-phenylindole (DAPI) to delineate normal DNA within a cell's nucleus

and digital images were taken using Nixon laser microscope, using 10x objective lens. Images were imported into Image Pro software (Media Cybernetics, USA). Blinded evaluation of TUNEL positive cells was undertaken using the software's smart function. Positive cells were counted in eight non-overlapping ROI in the frontal cortex on two sections from each animal. The ratio of TUNEL positive cells to total nuclei (DAPI stained cells) was calculated per ROI. These values were averaged to give the average ratio per section/animal. Quantification of Image Pro smart tool for identification of TUNEL positive cells was validated via manual counting four ROI in the frontal cortex of five animals.

1.4. Gene expression analysis

Frontal cortex brain tissue in D4, 7 and 21 female rats was used for quantitative RT- qPCR. Total RNA was extracted using the RNeasy plus Micro kit (Qiagen, West Sussex, UK) and protocol based on the single step acidified phenol-chloroform homogenisation method (221). RNA purification was quantified with the Nanodrop 1000 (Thermo Fisher Scientific, USA) and ND-1000 V3.8.1 software (NanoDrop Technologies, Wilmington, USA). All samples were normalised to 1 ng μL^{-1}. Reverse transcription was performed using the High Capacity RNA-cDNA kit (Applied Biosystems, Thermo Fisher Scientific, USA) and amplified using a Touchgene Gradient thermocycler (Teche Inc, Bibby Scientific Limited, Staffordshire, UK). Taqman probes (Applied Biosystems, Thermo Fisher Scientific, USA) or SYBR Green gene expression assays (BIO-RAD, Watford, UK) with rat-specific oligonucleotide primers (Sigma, Gillingham, UK) (Supplementary table 1) were used to analyse genes regulating inflammatory, apoptotic, unfolded protein response and GFAP through quantitative PCR using the Step One Plus q-PCR system and v2.2 software (Applied Biosciences). The GeNorm

algorithm was used against two reference genes RP29 and RPL13a (stability value M = 0.22) to determine gene expression.

1.5. Serum analysis

Blood was taken from neck veins post-mortem and allowed to clot at room temperature for approximately 30 minutes. Samples were centrifuged at 2000G for 10 minutes to allow separation of serum from cells. The serum was subsequently stored at -80 ^{o}C. Prior to use serum was thawed on ice. Concentrations of cortisol (Detect X Corticosterone Enzyme Immunoassay, Abor Assays, USA) and S100B (S100B (Human) ELISA Kit, Abnova, UK) were measured using manufacturers' instructions for D4 and 21 female rodents.

1.6. Statistical analysis

Data was analysed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA). Data were analysed using Mann U-Whitney test with statistical significance set at $p<0.05$. Gene expression data are expressed as mean $\pm$ SEM.

Supplementary table 1: Identifier of probe assays used for qPCR

Gene	Assay ID
CCL2 (Thermo Fischer)	Rn00580555_m1
Rps29 (Thermo Fischer)	Rn00820645_g1
Nfkb1 (Thermo Fischer)	Rn01399572_m1
ATF6 (Thermo Fischer)	***Rn01490844_m1***
Hspa5 (thermo Fischer)	***Rn00565250_m1***
ddit3 (Thermo Fischer)	***Rn00492098_g1***
CASP3 (Thermo Fischer)	***Rn00563902_m1***
GFAP (Thermo Fischer)	***Rn01253033_m1***
IL-6 (BioRad)	qRnoCIP0046986
RPL13a (BioRad)	qRnoCEP0050813
IL1b (BioRad)	qRnoCIP0026511
TNF- alpha (BioRad)	qRnoCEP0030948
TrL4 (BioRad)	qRnoCEP0024776

Appendix 2

Adapted TRIPS Study protocol

Minimising the adverse physiological effects of transportation on the premature infant

Final Version 1.0
25/6/18

Short title: TRiP Study

Acronym: TRiP

Study Registration:

ISRCTN:

IRAS Project ID: 236562

Study Sponsor: University of Nottingham

Sponsor reference: 18015

Funding Source: Invention for Innovation-NIHR

STUDY / STUDY OBJECTIVES AND PURPOSE

Hypothesis

Exposure to vibration and noise during inter-hospital transfer of preterm infants <32 weeks' gestation within the first 72 hours of life will adversely impact on measures of neonatal stress and result in micro neurological injury.

PRIMARY OBJECTIVE

The primary aims of the study are to quantify:

(i) the level of both vibration and noise as experienced by a preterm infant during inter-hospital transportation in ground ambulance in the UK
(ii) the physiological and biochemical changes that occur as a result of ambulance transportation
(iii) micro brain injury through measurement of urinary S100B and other biomarkers
(iv) development of intraventricular haemorrhage on cranial ultrasound

Secondary aims of the study are to monitor vibration and sound exposure, using a prototype measuring system, during neonatal transport using both a manikin and a small cohort of neonatal patients. In addition, we will evaluate vibration and sound exposure levels using an updated transportation system modified to reduce effects.

STUDY MANAGEMENT

The chief investigator, Dr Don Sharkey (Clinical Associate Professor in Neonatal Medicine, University of Nottingham) will ensure the study is run to Good Clinical Practice and study investigators on the project will also include Dr Lara Shipley, Paediatric Clinical Research Fellow and an as yet appointed successor. Dr Lara Shipley (and successor) will be undertaking the majority of the study data collection and analysis under the supervision of Dr Sharkey. Engineering input will be from Professor Donal McNally based at the University of Nottingham. Dr Shipley will report weekly progress of the study including adverse events to the chief investigator.

Eligibility criteria

Transported group- Preterm infants requiring transfer into or between NUHs neonatal units.

Inborn group- Preterm infants born at one of the NUH centres and requiring admission for on-going care.

Inclusion criteria

Phase 1 Transported group

- Transported infants <32 weeks' gestation
- Transportation within the first 72 hours of life
- Written parental consent

Phase 1 Inborn Group

- Infant < 32 weeks' gestation
- Less than 72 hours of age
- Written parental consent

Phase 2 Transported Group

- Neonatal unit infant of any age
- Written parental consent

Exclusion criteria (all participants)

- Lethal and/or major congenital abnormality known at study entry
- No realistic prospect of survival
- No informed consent
- Maternal death

STUDY / STUDY TREATMENT AND REGIMEN

Infants meeting study criteria will be recruited into either the transported or inborn group depending on their normal clinical course.

Data collection

All Phases

In addition to study data, routine clinical data will be recorded from the baby's notes during the study for later analysis on all infants. This will include:

- Demographic details (gestation, birth weight, gender, maternal ethnicity and maternal age)
- Pregnancy details (Maternal smoking, infection risk factors, medication, drug use, antenatal scan results, maternal antepartum haemorrhage, antenatal steroids, magnesium sulphate and pregnancy related complications)
- Delivery and resuscitation details (Mode of delivery, cord gases, resuscitation requirements, Apgar scores and neonatal admission observations)
- First routine cranial ultrasound scan results within the first 24 hours of life. This will be prior to transportation in the transported infant group and maybe performed by a trained clinical researcher if not done by the referring unit upon recruitment. In addition, subsequent routine cranial ultrasound results will be collected. These ultrasound images are routinely performed as part of the normal care pathway for these babies. The clinical team will store these images according to their current clinical practice (at NUH). The study team will review cranial ultrasound images to determine any evidence of brain injury or IVH for study purposes; this will not alter the normal discussions the clinical team caring for the baby have with the family.

At study entry for all babies.

- Collection of urine sample from infant using a urine bag or cotton wool balls within the nappy for later biomarker analysis (for example S100B).
- Baseline observations (e.g. temperature, HR, RR and oxygen saturations)
- We will collect routine blood gas data taken by the clinical team in addition to ventilator details, settings and measurements where appropriate.
- Where possible, a cranial ultrasound will be performed in transported infants (if not already done) prior to transfer.

During transportation (for the transported groups in phase 1 and 2)

- Temperature (normal care monitoring), HR, RR, oxygen saturations and cerebral regional saturation monitoring (using NIRS) will be collected.
- Vibration and noise levels using accelerometer sensors coupled to a vibration data logger (see diagram) and a noise dosimeter.
- GPS tracking to evaluate road route and speed of ambulance
- Real time video monitoring of the infant inside the incubator to allow evaluation of the infant's head movement during transfer with "vibration shocks" from the road surface/bumps and correlation of vibration with physiological changes. The images will be downloaded to the research database at the earliest opportunity and labelled with the study number only.
- Incubator vibration measurement using a smartphone application for comparison and quantification against purpose built vibration meters
- Data from blood tests and clinical investigations taken by the clinical team will also be collected.

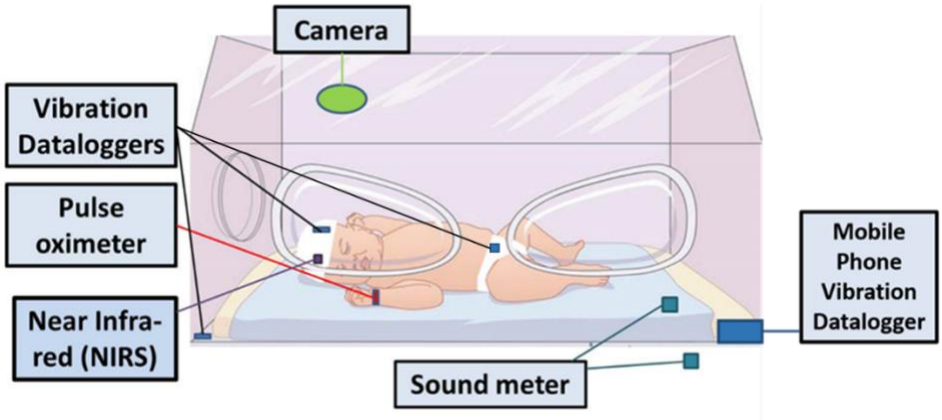
<u>Inborn Infants (Phase 1)</u>

- Temperature, HR, RR, oxygen saturation and NIRS monitoring from the point of enrolment into the study for up to the first 48 hours.
- Vibration and noise monitoring from the point of enrolment for up to the first 48 hours after arriving on the NICU.

Following either ambulance transport or recording on NICU, further urine samples will be collected at about 24, 48 and 72 hours after recruitment as previously described. This, along with the initial sample will be assayed at a later date to quantify biomarkers of stress (e.g. S100B or cortisol).

During transports when the research team are present, if the incubator is not is use for a patient (for example on the way to another hospital to transferring a patient) we will replicate the vibration and noise collection using a suitable newborn manikin with different mattress/restraint systems and our prototype sensors.

Outline of sensor set up during monitoring for both phase 1 and 2



All equipment will have appropriate checks, safety testing and sign off for use with NHS patients by the NUH Clinical Engineering Department prior to use.

For Phase 1, the data-logger, sound meter and vital sign monitors will be safely secured to the transport system in accordance with standard clinical procedures. The pulse oximetry and NIRS will be placed onto the infant's wrist and forehead using the provided self-adhesive sensors used in routine neonatal care. The vibration logger will be either attached to the material of the infant's hat or on top of a protective duoderm patch on the baby's forehead to provide skin protection, and therefore not in direct contact with the skin. The sound meters will not be in direct contact with the infant and will be secured either inside the incubator or transport trolley. A mobile phone will be connected to the incubator.

For Phase 2, the sensor set up will be as above, however, data loggers will be replaced with the prototype calibrated vibration and noise monitoring systems placed on both the infant's hat/nappy and at various interest points of vibration transmission (e.g. at the junction of the trolley frame and incubator) on the incubator and trolley itself. These sensors will not be in direct contact with the infant's skin and will be securely attached to the trolley itself. The transport incubator will be a new type of incubator and so is different from that in Phase 1.

Outcome data
The following on-going care and outcome data will be collected on each baby:

Subsequent level of care including cardiorespiratory support, infections, surgical treatment, medication, neuroradiological imaging, and health at discharge from hospital. For surviving babies this information will be collected until they are discharged from hospital. The same information will be collected until the date of death for non-surviving babies.

Appendix 3

Submitted Full Papers for PhD Thesis

Paper 1

Shipley L, Hyliger G and Sharkey D. Temporal trends of in-utero and early postnatal transfer of babies born 23^{+0} to 27^{+6} weeks' gestation between 2011-2016: A UK population study. (Draft Paper formatted for journal submission)

Temporal trends of in-utero and early postnatal transfer of babies born 23^{+0} to 27^{+6} weeks' gestation between 2011-2016: A UK population study

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Objective

Extremely preterm infants have an increased risk of brain injury following postnatal transport (PNT). We aimed to explore trends of in-utero transfer (IUT) and early PNT

Design

Retrospective cohort study using national data.

Setting

UK neonatal units

Patients

Infants 23^{+0} to 27^{+6} weeks' gestation admitted to UK neonatal units

Main Outcome

Primary outcome was the prevalence of IUT and PNT within 72 hours of life in the UK. Secondary outcomes included mortality and inter-hospital transfer level. Temporal changes were compared across two equal epochs 2011-2013 (Ep1) and 2014-2016 (Ep2).

Results

14719 infants were included (Ep1=7363 and Ep2=7256). IUTs significantly decreased over time from 28.3% (Ep1=2089) to 26.0% (Ep2=1916) (OR 0.90, 95% CI 0.84-0.97, p<0.01). Conversely, PNTs increased from 19.8% (Ep1=1416) to 21.5% (Ep2=1581) (OR 1.11, 95% CI 1.02-1.20, p=0.01). Level 3 to level 3 PNTs increased by 35% from 8.1% (Ep1=119) to 10.2% (Ep2=161, p=0.05). Deaths decreased from 21.6% (Ep1=1592) to 19.3% (Ep2=1421) (OR 0.90, 95% CI 0.83-0.97, p=0.01). Infants born in a level 3 centre had significantly reduced mortality compared to birth in a non-tertiary centre, with greatest reduction seen in infants <24 weeks gestational age.

Conclusions

IUTs have significantly decreased in the UK with a parallel increase in PNT, especially between level 3 to level 3 hospitals. To achieve the government ambition of a 50% reduction in newborn brain injury by 2025, urgent review of IUT service configuration and neonatal capacity is needed if we are to meet ambitious government targets and improve outcomes for these high-risk babies.

Introduction

In 2003, UK neonatal services re-organised into managed clinical networks leading to the development of a tiered hospital system consisting of different specialist levels of care working together with the aim to improve provision of quality care and neonatal outcomes (1). Following implementation of centralised care, national guidance was developed advising extremely preterm infants should be delivered within and cared for in hospitals with a level 3 NICU on site due to their increased risk of mortality and severe morbidity (2, 3). This has led to a significant reduction in mortality, although this improvement was not seen in survival without neurodisability (4-6).

Extremely preterm infants who are either born in or undergo in-utero transfer (IUT) into a level 3 centre have reduced mortality and morbidity compared to those who undergo early postnatal transfer (PNT) (4, 7-9). Evidence supporting better outcomes in high-risk infants who undergo IUT, compared to early PNT, into level 3 centres has resulted in the adoption of IUT as the optimal standard of care where feasible (10). However, due to maternal clinical instability, precipitous delivery and barriers to IUT (11), early PNT can be unavoidable. Early PNT of extremely preterm infants has been associated with an increased risk of severe IVH (12-15). Infants with a severe IVH have increased mortality and an estimated 50 to 80% of survivors develop cerebral palsy or cognitive impairment (16). Not only does this have a significant cost to society (14, 17), but it has a long-term impact on the quality of life of the child and their family. However, even infants with low grade IVH (1 or 2) have lower neurodevelopmental scores compared to those without IVH (16, 18). As such, strategies to reduce this level of morbidity are of great importance to public health and a key driver for government plans.

The cause of excess severe brain injury in PNT infants <28weeks gestational age (GA) remains unclear but it is likely to be multifactorial. The perinatal period is a high-risk time for IVH in these babies with most occurring in the first few days of life (19, 20), a time when they are most likely to undergo PNT (21). In the UK, approximately 450 infants <27weeks GA are transferred in the first 3 days of life. It has been proposed that the transport process itself may contribute to this increased morbidity seen in these infants due to the exposure to an adverse environment, such as excessive noise, vibration and temperature instability (22-26). Excessive vibration exposure has been associated with poor health in adults (27, 28) and brain injury in animal studies (29, 30).

In 2019, the National Health Service updated their long-term plan with the aim to achieve a 50% reduction in stillbirths, maternal mortality, neonatal mortality and serious brain injury by 2025 (3). To achieve this ambitious target, multiple areas and strategies will need to be explored, including the care of extremely preterm infants who have a high risk of mortality or significant brain injury (31-33). Understanding the prevalence of IUT and PNT in the UK and how these are changing over time could provide invaluable data for service optimisation and potential strategies to improve IUT. Gaining an appreciation of same level hospital transfers could provide information on cot capacity issues, which is required to avoid unnecessary exposure of these infants to PNT.

The primary aim of this study was to establish the prevalence of infants 23^{+0} to 27^{+6} weeks GA who undergo either IUT or PNT. The secondary aims were to evaluate changes over time, describe referring to receiving hospital levels and associated mortality rates.

Methods

Study Design and participants

This is a retrospective cohort study of prospectively routinely collected clinical data obtained from the National Neonatal Research Database (NNRD). The NNRD is a validated database containing data on demographic details, antenatal care and postnatal outcomes on all neonatal admissions in the UK.

Data were collected on all infants born 23^{+0} to 27^{+6} weeks' gestation that were admitted to neonatal units in England, Scotland and Wales between 2011-2016. Infants were identified as undergoing PT within 72 hours of life the data fields "Discharge destination", "Admission time" and "Discharge time". IUT infants were identified if their booking hospital code did not match the place of birth hospital code. Hospital care level was determined from the "Place of birth NHS code" data field.

Infants born at home, with missing hospital codes (therefore IUT status cannot be determined), missing hospital admission episodes and those with erroneous data were excluded. Ethical approval was given by the London-City and East Research Committee (REC: 17/LO/1822).

Outcomes

The primary outcome was to establish the prevalence of IUT and PT within 72 hours of age for infants 23^{+0} to 27^{+6} weeks gestational age. The secondary outcomes were mortality rates over the study periods and hospital transfer levels between referring and receiving units.

Statistical Analysis

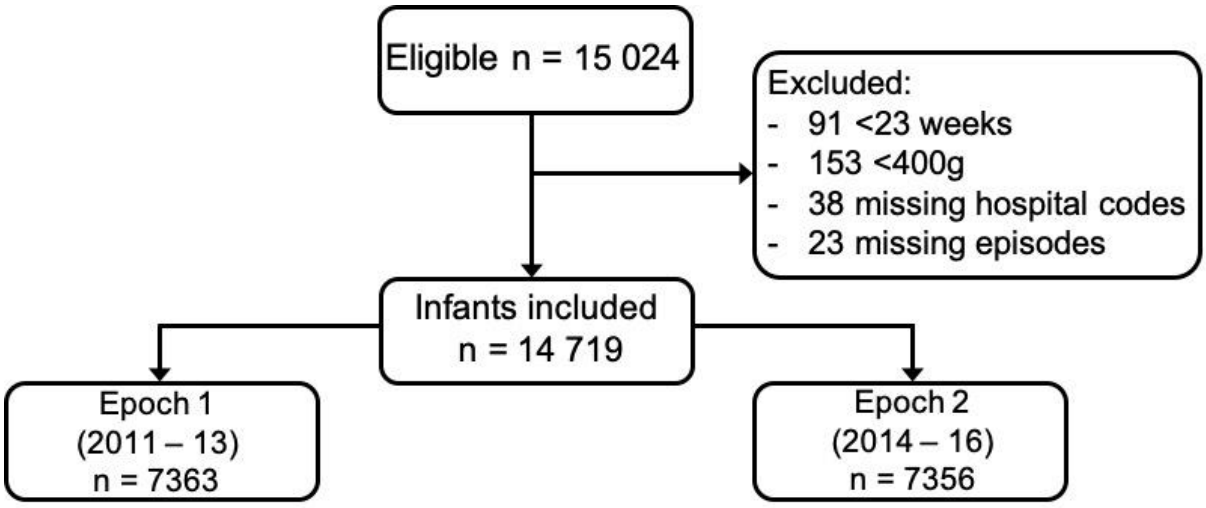
The population was separated into two equal epochs (Ep1 from 2011-2013 and Ep2 from 2014-2016) to evaluate temporal changes. Mann-Whitney U test was used for group

analysis to compare changes between epochs. Odds ratio (OR) and confidence intervals (CI) were calculated with significance set as $p<0.05$. Statistical analysis was performed using Stata SE (StataCorp, Version 15).

Results

During the study period, 14 719 infants born 23^{+0} to 27^{+6} weeks' gestation were admitted to neonatal units in England, Scotland and Wales. Of these 7363 were in Ep1 and 7356 in Ep2 (Figure 1).

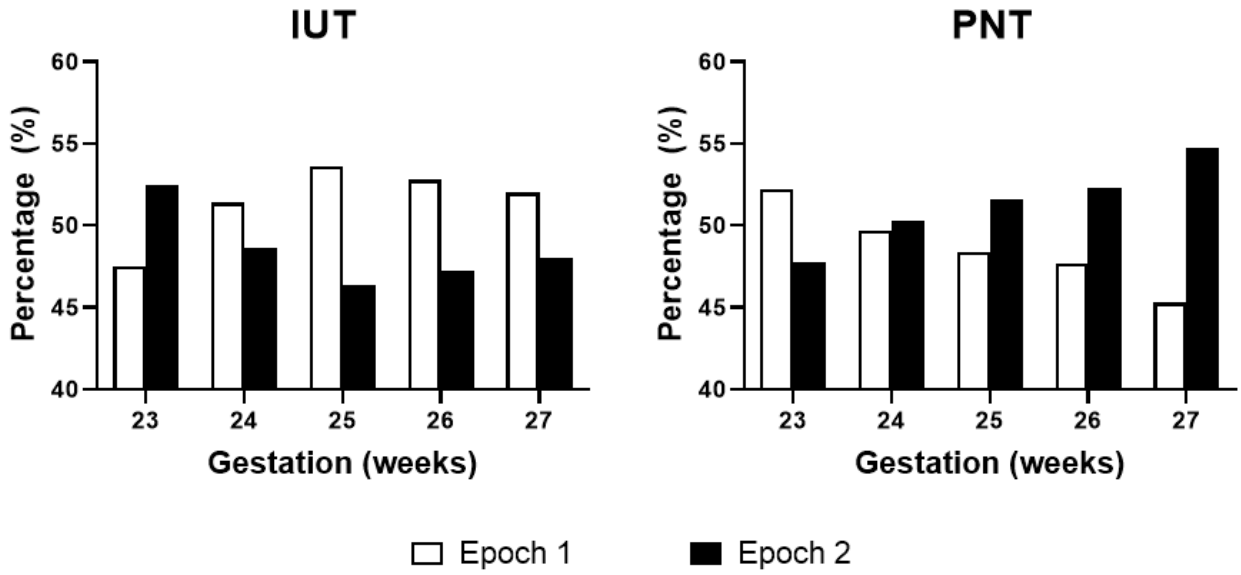
Figure 1. Flowchart of study population demonstrating included/excluded infants 23^{+0} to 27^{+6} weeks gestational age admitted to neonatal units in the UK



A total of 4005 (27%) infants underwent IUT with a significant decrease in numbers between epochs from 28.3% to 26% (Ep1=2089 and Ep2=1916; OR 0.90, 95% CI 0.84-0.97, $p<0.01$). Conversely, 3042 (20.7%) infants had early PNT, a significant increased between epochs from 19.8% to 21.5% (Ep1=1461 and Ep2=1581; OR 1.11, 95% CI 1.02-1.20, $p=0.01$). The

total percentage of all IUT and PNTs by GA between the two epochs are shown in Figure 2 and Supplementary Table 1.

Figure 2. Comparison of in-utero (IUT) and postnatal transfers (PNT) within 72 hours of life between Epoch 1 (2011-2013) and Epoch 2 (2014-2016) by GA (IUT n=4005, PNT n=3042)



Overall, Level 2 to Level 3 transfers were most prevalent accounting for 63.3% (n = 1924), an increase between epochs of 13.3% (Table 1). The early PNT of infants between Level 3 NICUs has seen the greatest proportional increase of 35% from Ep1 to Ep2. There were no difference in median gestational age (Ep1 26 weeks (IQR 25-27) and Ep2 26 weeks (IQR 25-27), p=0.30) or birthweight (Ep1 890g (IQR 750-1000), Ep2 845g (715-956), p=0.18) between level 3 to level 3 transferred infants over time (Supplementary Table 2).

Table 1. Referring and receiving neonatal unit levels for all postnatally transported infants 23^{+0} to 27^{+6} weeks' gestation within 72 hours of age in the UK

Unit level		**Epoch 1 n = 1461**	**Epoch 2 n = 1581**		
Referring	**Receiving**	**PT infants n (%)**	**PT infants n (%)**	**% change**	**p value**
L3	L3	119 (8.1)	161 (10.2)	+ 35.3	0.05
L2	L3	902 (61.7)	1022 (64.6)	+13.3	0.09
L1	L3	317 (21.7)	279 (17.6)	-12.0	<0.01
L3	L2	25 (1.7)	31 (2.0)	+24.0	0.61
L2	L2	36 (2.5)	32 (2.0)	-11.1	0.41
L1	L2	34 (2.3)	22 (1.4)	-35.3	0.06
MLU	L2	2 (0.1)	0 (0)	n too small	-
MLU	L3	16 (1.1)	14 (0.9)	n too small	-
Missing	Missing	9 (0.6)	19 (1.2)	-	-

L3, Level 3; L2, Level 2; Level 1; MLU, Midwife-led unit; PT, postnatal transport

Overall, there has been no significant difference in the proportion of infants who received a full course of antenatal steroids over time, irrespective of transportation subgroup (64.6% vs 65.3%, p=0.36). However, for infants who underwent PNT there has been a significant increase between Ep1 from 40% (n=578) and Ep2 at 43.3% (n=685) (p<0.01, Table 2).

Table 2. Comparison of antenatal steroid course between in-utero transferred and postnatally transported infants 23^{+0} to 27^{+6} weeks' gestational age between 2011 - 2016

	Epoch 1 (2011 – 2013)		Epoch 2 (2014 – 2016)	
	PNT n = 1461	**IUT n = 2089**	**PNT n = 1581**	**IUT n = 1916**
No steroids	365 (25.0)	87 (4.2)	268 (17.0)	57 (3.0)
Incomplete course	465 (31.8)	225 (10.8)	550 (34.8)	208 (10.9)
Complete course	578 (40.0)	1717 (82.2)	685 (43.3)	1597 (83.4)
Unknown/missing	53 (3.6)	56 (2.7)	78 (4.9)	54 (2.8)

Data are n (%)

Mortality

A total of 3013 (20.5%) infants died during the study period, with a significant decrease between epochs (Ep1=1592 (21.6%) vs Ep2=1421 (19.3%); OR 0.90, 95% CI 0.83-0.97, P=0.01). Infants born at 23 to 24^{+6} weeks GA showed the greatest benefit from birth in a level 3 hospital and had significantly reduced mortality compared to birth in either a level 1 or 2 hospital (Table 3). Following early PNT, 22.8% (n=686) of infants died. The proportion of these infants who died and were PNT from level 1 to level 3 units has decreased over time (Ep1 21.4% (n=78) vs Ep2 17.7% (n=57), p=0.22) but not significantly. Whereas infants PNT from level 2 to level 3 units has remained the same over time (Ep1 67.2% (n=242) vs Ep2 68.3% (n=218), p=0.92). However, deaths following PNT from level 3 and level 3 units has significantly increased between epochs (Ep1 5.6% (n=20) vs Ep2 9.7% (n=31); OR 1.83 (1.02-3.29), p=0.04, Supplementary table 3). There was no significant difference in demographic characteristics or antenatal steroid course between epochs for these infants.

Table 3: Comparison of births and death by place of birth hospital level per gestational week for infants 23^{+0} to 27^{+6} weeks gestational age born within the UK from 2011-2016.

Gestation (weeks)	Level 1 (n= 660)	Level 2 (n = 3804)	Level 3 (n = 10 053)
23			
Births	44 (6.6)	236 (6.2)	814 (8.1)
Deaths	30 (68.2)	156 (66.1)	400 (49.1)
24			
Births	111 (16.8)	548 (14.4)	1781 (17.7)
Deaths	51 (45.9)	207 (37.8)	589 (33.1)
25			
Births	122 (18.5)	633 (16.6)	2056 (20.4)
Deaths	28 (23.0)	147 (23.2)	416 (20.2)
26			
Births	180 (27.3)	879 (23.1)	2589 (25.8)
Deaths	30 (16.7)	122 (13.9)	367 (14.2)
27			
Births	203 (30.8)	1508 (39.6)	2813 (28.0)
Deaths	19 (9.4)	131 (8.7)	253 (9.0)

Data are n (%) of births at each unit level

Discussion

This study aimed to look at current national trends of IUT and PNT of infants 23^{+0} to 27^{+6} weeks' GA. We found the incidence of IUT has significantly decreased over time, whilst conversely early PNT within 72 hours have increased. This UK (excluding Northern Ireland) population study is the largest to date to evaluate the current trends in the centralised approach to management of extremely preterm infants. There are several barriers to undertaking IUT, such as length of time required by staff with other roles, lack of available neonatal and maternal beds within the same hospital and concerns regarding imminent delivery (11). Careful consideration is required to determine which women can be safely transferred using obstetric expertise and predictors of preterm delivery (34, 95). However,

evidence suggests the majority of women in threatened preterm labour do not deliver within 24 hours of presentation (36 – 38). Additionally, infants rarely deliver during transportation (34, 36, 39). This highlights the window of opportunity for IUT may be greater than currently perceived. The lack of detailed clinical background information for infants who had early PNT limited our ability to assess whether there were missed opportunities for IUT. However, our finding of a significant increase in the number of early PNT infants who received a full course of antenatal steroids, would suggest the mothers of these infants were an inpatient at the referring centre for at least a period of 24 hours prior to delivery, during which an IUT may have been feasible.

The significant increase in early PNTs could expose a greater proportion of vulnerable infants to an increased risk of severe IVH (12-14). The reduction in successful IUTs may be in part due to difficulties undertaking the IUT pathway, as unlike for neonatal transport provision, a co-ordinated centralised network service has yet to be developed. Additional pressure from lack of cot capacity at level 3 units is likely to contribute to the need for PNT (40). Our finding that there has been a significant increase in level 3 to level 3 transfers over time, could be a reflection of cot capacity issues.

Our results are consistent with previous studies (4, 8) demonstrating improved survival with birth in a level 3 hospital compared to at a level 1 or 2 centre. The benefit was greatest for those at the extremes of viability. The recent BAPM framework (10), advocates a change in practice towards more proactive management of infants at the lowest gestational ages, following improved survival rates in these infants with advancing perinatal care. Although we were unable to account for bias, such as lack of antenatal steroid use in outborn infants

due to imminent delivery; our study, highlights the necessity of strategies to improve the IUT pathways for these infants to improve outcomes where possible.

The gestational split on IUT and PNT suggest that an increasing number of infants at 23 weeks GA are undergoing IUT. However, the pattern appears reversed for all other gestations, a worrying trend considering this makes up the greatest proportion of infants <28 weeks. The significant increase in mortality for infants who underwent level 3 to level 3 transfers is concerning. Given these infants should have been receiving the same standards of care at both centres and there was no difference in demographic background or antenatal steroid administration, this raises the concern regarding early PNT as a contributing factor in this high-risk group.

Strengths of study

A major strength of this data is the large number of infants included using prospectively collected data from all neonatal units in the UK. This enables evaluation of trends for the whole population at national level and allows for variation in management across neonatal networks. Since data is prospectively entered on a daily basis, this allows accurate evaluation of timing of PNT and changes in outcomes over time.

Limitations

The main limitations of our study include the lack of clinical details on the cause of death and potential contributing factors. Another limitation is the lack of PNT details beyond coding within the database, this would be useful to explore the reasons for PNT over IUT and whether there was a missed opportunity. However, we tried to mitigate this by

evaluation of antenatal steroid use which could suggest there was sufficient time to undertake IUT.

A further limitation to our study is due to the assumption that all infants born in a different centre to where they were booked were IUTs, as the NNRD does not directly record IUT. We acknowledge the overall number is likely to be less due to transfer of care to a level 3 centre for clinical reasons during the pregnancy or the mother could have been visiting another region in the UK (e.g. on holiday) prior to delivery. However, the number of these infants is likely to remain unchanged over time, therefore the overall trend is unlikely to change. We also acknowledge that we were unable to account for the number of mother's who were IUT but did not subsequently deliver and infants who were IUT and died in the delivery room as these data are not recorded within the NNRD.

In addition, the NNRD only records infants who survive to neonatal unit admission. Infants at the extremes of gestational age who do not have IUT are more likely not to be offered active care at non-tertiary centres or die in the delivery room. We were unable to account for these deaths in our study, whereas those infants at the lowest gestational ages who underwent IUT to a tertiary centre are more likely to have active management, successful stabilisation following birth and admission to NICU.

Although the NNRD is a validated database and uses strict measures to ensure erroneous data is minimised, it relies on clinicians to input data. Therefore, we acknowledge some data entry error may remain.

Conclusion

This UK population study highlights, despite the national recommendation of IUT, the prevalence of IUT has decreased over time. Consequently, the number of early PNT has increased, especially between level 3 to level 3 hospitals. There is increasing evidence that early PNT exposes infants to an adverse environment and is associated with risk of severe IVH. It is important service providers address factors, such as staff shortages, which contribute to lack of cot capacity or midwifery staff able to undertake IUT. Future work should focus on strategies to increase the proportion of IUTs in the UK through development of guidelines and education to aid recognition of which women could undergo IUT and referral for IUT earlier. Further evaluation of the potential benefits of a centralised referral system alongside up to date maternity bed and cot status to facilitate the co-ordination of this care pathway is required. These interventions could reduce unnecessary early PNTs and the risk of associated adverse outcomes to achieve the UK governments ambitious targets.

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Supplementary Online Content

Temporal trends of in-utero and early postnatal transfer of babies born 23^{+0} to 27^{+6} weeks' gestation between 2011-2016: A UK population study

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Supplementary Table 1: Prevalence of in-utero and postnatal transportation for infants 23^{+0} to 27^{+6} weeks gestational age born between 2011 and 2016 in the UK

	Epoch 1 (n = 7363)		Epoch 2 (n = 7356)	
Gestation (weeks)	**IUT (n = 2089)**	**PNT (n = 1461)**	**IUT (n = 1916)**	**PNT (n = 1581)**
$23 – 23^{+6}$	124 (1.7)	121 (1.6)	137 (1.9)	111 (1.5)
$24 – 24^{+6}$	391 (5.3)	295 (4.0)	370 (5.0)	298 (4.1)
$25 – 25^{+6}$	470 (6.4)	338 (4.6)	407 (5.5)	360 (4.9)
$26 – 26^{+6}$	560 (7.6)	381 (5.2)	500 (6.8)	418 (5.7)
$27 – 27^{+6}$	544 (7.4)	326 (4.4)	502 (6.8)	394 (5.4)

IUT, In-utero transfer; PNT, Postnatal transportation

Supplementary Table 2. Gestational age of infants who were postnatally transferred from level 3 to level 3 hospitals within 72 hours of age over time

Gestation (weeks)	**Total infants (n= 280)**	**Epoch 1 (n = 119)**	**Epoch 2 (n = 161)**
23	10 (3.5)	6 (5.0)	4 (2.5)
24	40 (14.3)	13 (10.9)	27 (16.8)
25	48 (17.1)	17 (14.3)	31 (19.3)
26	83 (29.6)	38 (31.9)	45 (28.0)
27	99 (35.4)	45 (37.8)	54 (33.5)

Data are n (%)

Supplementary Table 3. Demographic characteristics of infants 23^{+0} to 27^{+6} weeks' gestational age who underwent early level 3 to level 3 postnatal transfer and died

Variables	Total infants (n = 51)[a]	Epoch 1 (n = 20)[a]	Epoch 2 (n = 31)	P value[b]
Gender (male)	32 (62.7%)	10 (50%)	22 (71%)	0.13
Birth weight (grams)	768 (630-880)	772 (573-895)	768 (630-855)	0.85
Gestation (weeks)				0.66
23	7 (13.7)	4 (20.0)	3 (9.7)	
24	13 (25.5)	5 (25.0)	8 (25.8)	
25	9 (17.6)	2 (10.0)	7 (22.6)	
26	9 (17.6)	3 (15.0)	6 (19.4)	
27	13 (25.5)	6 (30.0)	7 (22.6)	
Antenatal Steroids				0.08
None	8 (16.0)	2 (10.)	6 (20.0)	
Incomplete	11 (22.0)	2 (10.0)	9 (30.0)	
Complete	29 (58.0)	14 (70.0)	15 (50.0)	
Missing	2 (4.0)	2 (10.0)	0	
Age at transfer				<0.01
<24 hours	29 (56.9)	14 (70.0)	15 (48.4)	
24-48 hours	7 (13.7)	3 (15.0)	6 (19.4)	
48-72 hours	15 (29.4)	3 (15.0)	10 (32.3)	
Day of Death				<0.01
0-3 days	6 (11.8)	4 (20.0)	2 (6.5)	
3-7 days	11 (21.6)	2 (10.0)	9 (29)	
7-14 days	12 (23.5)	5 (25.0)	7 (22.6)	
14-21 days	5 (9.8)	3 (15.0)	2 (6.5)	
>21 days	17 (33.3)	6 (30.0)	11 (35.5)	

[a] Data are n (%) or median (interquartile range)

[b] Categorical data analysed using chi squared test, non-normally distributed continuous data analysed using Mann-Whitney U test

Paper 2

Shipley L, Gyorkos T, Dorling J, Tata L, Szatkowski K and Sharkey D. Risk of Severe Intraventricular Haemorrhage in the First Week of Life in Preterm Infants Transported Before 72 hours of age. Pediatr Crit Care Med. 2019;20(7):638-44

Risk of Severe Intraventricular Hemorrhage in the First Week of Life in Preterm Infants Transported Before 72 Hours of Age*

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Objectives: Evaluate the risk of severe intraventricular hemorrhage, in the first week of life, in preterm infants undergoing early interhospital transport.

Design: Retrospective cohort study.

Setting: Tertiary neonatal centers of the Trent Perinatal Network in the United Kingdom.

Patients: Preterm infants less than 32 weeks gestation, who were either born within and remained at the tertiary neonatal center (inborn), or were transferred (transported) between centers in the first 72 hours of life.

Interventions: None.

Measurements and Main Results: Multivariable logistic regression models adjusting for key confounders were used to calculate odds ratios for intraventricular hemorrhage with 95% CIs for comparison of inborn and transported infants. Cranial ultrasound findings on day 7 of life. Secondary analyses were performed for antenatal steroid course and gestational age subgroups. A total of 1,047 preterm infants were included in the main analysis. Transported infants (n = 391) had a significantly higher risk of severe (grade III/IV) intraventricular hemorrhage compared with inborns (n = 656) (9.7% vs 5.8%; adjusted odds ratio, 1.69; 95% CI, 1.04–2.76), especially for infants born at less than 28 weeks gestation (adjusted odds ratio, 1.83; 95% CI, 1.03–3.21). Transported infants were less likely to receive a full antenatal steroid course (47.8% vs 64.3%; $p < 0.001$). A full antenatal steroid course significantly decreased the risk of severe intraventricular hemorrhage irrespective of transport status (odds ratio, 0.33; 95% CI, 0.2–0.55). However, transported infants less than 28 weeks gestation remained significantly more likely to develop a severe intraventricular hemorrhage despite a full antenatal steroid course (adjusted odds ratio, 2.84; 95% CI, 1.08–7.47).

Conclusions: Preterm infants transported in the first 72 hours of life have an increased risk of early-life severe intraventricular hemorrhage even when maternal antenatal steroids are given. The additional burden of postnatal transport could be an important component in the pathway to severe intraventricular hemorrhage. As timely in-utero transfer is not always possible, we need to focus research on improving the transport pathway to reduce this additional risk. (*Pediatr Crit Care Med* 2019; 20:638–644)

Key Words: cerebral intraventricular hemorrhage; infant, preterm; newborn; perinatal care; transport

*See also p. 677.

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Centralization of neonatal intensive care has improved preterm mortality; however, morbidity such as neurodisability remains unchanged (1). Preterm infants have an increased risk of intraventricular hemorrhage (IVH) (2, 3); although the etiology is multifactorial, the principle cause is due to the fragility of the germinal matrix and fluctuations in cerebral blood flow (4). However, this risk can be significantly reduced by administration of antenatal steroids (ANS) prior to delivery (5). Severe IVH (grades III and IV) is associated with increased mortality and an estimated 70% of survivors develop cerebral palsy or cognitive impairment (6). This has a significant impact not only on quality of life but on society with an estimated lifetime cost per child with cerebral palsy of $1.3 million (£1 million) (7, 8). Furthermore, infants with mild IVH have lower developmental scores compared with those with no IVH (6, 9).

The majority of IVHs start within the first hours and days of life (3), coincident with the period when interhospital transport frequently occurs (10) and, in many instances, grade 1 or 2 hemorrhages extend to the more severe grade (11). This is of particular concern as one in six preterm infants less than 32 weeks gestation (~1,400 infants) born in the United Kingdom are transported in the first 72 hours of life (10) and up to one in five are transported in Canada (12). The EPICure 2 study found only 7% of extremely preterm infants transported on the first day of life, survived without significant morbidity, lower than those born and cared for in level 2 (17%) and level 3 units (15%) (1). Many historical cohort studies have reported an increased risk of severe IVH with postnatal interhospital transport (8, 13–15), but others have not (16, 17). These have a number of limitations including exclusion of high-risk patients and omission of important confounders including the role of ANS as a potentially neuroprotective agent and proxy of good quality antenatal care. More importantly, none account for the lack of differentiation between early perinatal (in the first week) and later brain injury; the latter could be attributed to other risk factors associated with longer-term neonatal care.

A recent Canadian study, of almost 3,000 infants less than 29 weeks, found outborn (transported) infants were more likely to have a poor neurodevelopmental outcome at 2 years old (12). The authors postulated that the outborn infants were sicker initially but not 12 hours after transfer and suggested the illness severity after delivery and during transport may have a major impact on these outcomes. However, no data were presented on the day of transfer or risk of severe IVH in the first week of life, which could better reflect early perinatal risks including interhospital transport.

Understanding the prevalence of IVH and the associated perinatal factors in the first week of life could provide useful data for studies aimed at reducing this during the period of greatest risk. Our primary aim was to evaluate the relationship between neonatal transport, early severe IVH, and ANS administration in preterm infants born less than 32 weeks gestation who were transferred within the first 72 hours of life.

MATERIALS AND METHODS

Study Design and Participants

This retrospective cohort study used prospectively collected anonymized clinical data from a validated online national U.K. database (18), BadgerNet (Clevermed, Edinburgh, United Kingdom), between 2007 (the start of the database in this network) and 2016 as well as local clinical records where appropriate. Data were collected on all preterm infants born less than 32 weeks gestational age (GA), who were either born in (inborn) or transferred into (from regional centers of any care level) or between one of the two Nottingham University Hospitals (NUHs) tertiary neonatal ICUs (NICU). These are the two regional tertiary referral centers for the U.K. Trent Perinatal Network.

BadgerNet creates a single record of care for every newborn admitted to the NICU and includes information on obstetric care and subsequent postnatal management. In order to assess IVH potentially related to transport within 72 hours of birth, we included infants who had a cranial ultrasound scan (CrUSS) on day 7 (± 1) after birth as per the standard tertiary center protocol; those who died or were transported out of NUH before this scan were excluded. Ethical approval was given by the School of Medicine Ethics Committee, University of Nottingham.

Outcome

The primary outcome was CrUSS findings taken on day 7 (±1) of life. All CrUSSs used a standardized protocol with a predefined series of anatomical views obtained. These were then evaluated by a consultant pediatric neuroradiologist or neonatal consultant and graded using the classification by Papile et al (19). Infants were categorized as having no IVH or any grade of IVH, which was further divided into no or mild IVH (grade 1 or 2) and severe IVH (grade 3 or 4).

Statistical Analysis

Infants were grouped according to transport status: inborn versus transported. The two groups were further divided into less than 28 week and 28–32 week GA subgroups. Initial assessment of the association between transport, within 72 hours of birth, and IVH outcome was conducted using a chi-square test. Associations between demographic and clinical variables with transport and with IVH were assessed using chi-square tests for categorical data and Mann-Whitney *U* test for nonnormally distributed continuous data.

Multivariable logistic regression was used to calculate the adjusted odds ratio (aOR) for the association between transport and IVH, controlling for confounding factors. A priori confounders (gender, gestation, and birth weight) were included in the logistic model in addition to any variable that had a statistically significant association with both exposure (transport) and outcome (IVH) at the 5% level. Variables that were not statistically significant on univariate testing were individually added back into the model and included as potential confounders if there was a change in the aOR for IVH in either direction by greater than or equal to 10%. Confounding factors evaluated for inclusion in the regression model were mode of delivery, intrauterine growth restriction (based on serial fetal ultrasound), maternal infection risk (maternal IV antibiotics or sepsis, prolonged rupture of membranes > 18 hr, maternal pyrexia, and Group B Streptococcus), antepartum hemorrhage, maternal recreational drug use, intubation at birth, surfactant administration, chest compressions at birth, delivery room adrenaline, Apgar scores at 1 and 5 minutes, and NICU inotrope treatment. Similar models were created with the main outcome severe IVH only, with the baseline group including those with no or mild IVH.

To evaluate whether ANS administration modified the effect of transport within 72 hours of birth on IVH, infants were stratified by whether they had received no or an incomplete course of ANS or a complete course of ANS at least 24 hours prior to delivery. Logistic multivariable models were also

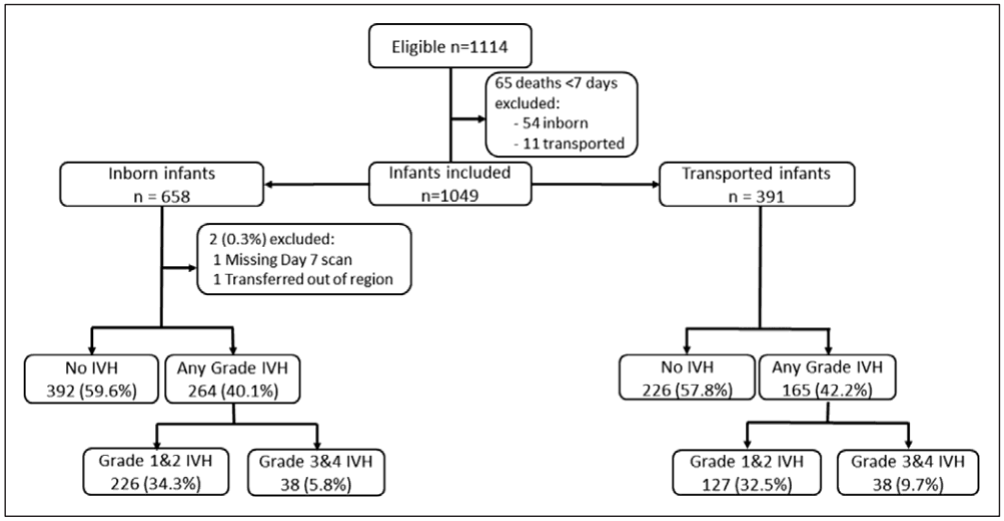

Figure 1. Flowchart of study participants demonstrating inclusions/exclusions and prevalence of intraventricular hemorrhage (IVH) for each cohort.

used to evaluate the association between transportation and IVH or severe IVH adjusting for confounding factors, depending on ANS status. All statistical analyses were performed using Stata SE (Version 14; StataCorp, College Station, TX).

RESULTS

A total of 1,114 preterm infants met the inclusion criteria (inborn n = 713 and transported n = 401; **Fig. 1**). Sixty-five infants died prior to day 7 CrUSS, one infant had a missing CrUSS, and one infant was transferred out of the network before day 7, leaving 1,047 for analysis. Extreme prematurity and respiratory causes were identified as the main factors contributing to the cause of death in infants who died before day 7 in both groups (**Table S1**, Supplemental Digital Content 1, http://links.lww.com/PCC/A933; **Table S2**, Supplemental Digital Content 2, http://links.lww.com/PCC/A934; and **Table S3**, Supplemental Digital Content 3, http://links.lww.com/PCC/A935). Both groups had similar median birth weights and GA at birth (**Table 1**). Overall, 51.9% (n = 203) of transfers occurred between level 3 units (n = 71 < 28 wk GA). Transported infants were more likely to be male, have a greater prevalence of maternal infection risk factors, and were more likely to be intubated or receive surfactant compared with inborn infants. Over the period of the study, the prevalence of mild and severe IVH did not change (data not shown).

Irrespective of transport group, infants less than 28 weeks GA were significantly more likely to develop any IVH (58.6% vs 25.4%; $p < 0.001$) and severe IVH (12% vs 3%; $p < 0.001$) compared with those born 28–32 weeks GA. The prevalence of any grade IVH was similar between transported (n = 165; 42.2%) and inborn infants (n = 264; 40.1%) and the OR was not statistically significant before (OR, 1.08; 95% CI, 0.84–1.4; p = 0.51) or following adjustment (aOR, 0.99; 95% CI, 0.75–1.35; p = 0.96). Overall, when including transfer status, transported infants were more likely to develop severe IVH compared with inborns (**Table 2** and **Fig. 2**). However, secondary analysis demonstrated this association only persisted for transported infants less than 28 weeks GA following multivariate logistical regression analysis when accounting for the variables listed in Table 1 (excluding ANS, which were included in subsequent analysis). For transported infants, 310 had pre-transfer CrUSS performed with 2.3% (n = 7) having a diagnosis of severe IVH, but by day 7 this proportion had increased to 9.7% (n = 38) compared with inborns (5.8%) (**Table 3**). Furthermore, of the 38 transported infants with severe IVH, 35 occurred in those transported in the first 48 hours of life with 27 of these infants less than 28 weeks GA (**Table S4**, Supplemental Digital Content 4, http://links.lww.com/PCC/A936).

For the assessment of ANS, 19 infants were excluded as they had missing data, leaving 1,028 for analysis. Of these, 610 infants (58.2%) received a full course of ANS (**Fig. S1**, Supplemental Digital Content 5, http://links.lww.com/PCC/A937; **legend**, Supplemental Digital Content 7, http://links.lww.com/PCC/A939), 228 (37.4%) were subsequently diagnosed with an IVH with 26 (4.3%) developing severe IVH. Inborn infants were significantly more likely to receive a full course of ANS compared with transported infants ($p < 0.001$) (Table 1). Irrespective of transport status, a full course of ANS was associated with a 33% decrease in odds of any IVH (OR, 0.67; 95% CI, 0.52–0.87; p = 0.003) and 67% reduction of severe IVH (OR, 0.33; 95% CI, 0.2–0.55; $p < 0.001$). Overall, transported infants who received no or an incomplete course of ANS were at increased odds of developing any IVH (OR, 1.47; 95% CI, 1.10–2.17; $p < 0.05$) although this was not statistically significant following multivariable adjustment (aOR, 1.37; 95% CI, 0.88–2.14).

Subgroup analysis of infants less than 28 weeks GA showed a complete course of ANS was associated with significantly reduced odds of both any grade IVH by 41% (OR, 0.59; 95% CI, 0.40–0.86; $p < 0.001$) and severe IVH by 63% (OR, 0.37; 95% CI, 0.21–0.66; $p <$ 0.001) irrespective of transport status. However, inclusion of ANS in the multivariable regression model demonstrated transported infants less than 28 weeks remained significantly more likely to have severe IVH despite a full course of ANS (**Fig. 3** and **Table 4**). Transported infants 28–32 weeks GA, irrespective of maternal ANS treatment, had a greater proportion of severe IVH overall (2.3% vs 1.2%), but this was not statistically significant (Table 4; Fig. S1, Supplemental Digital Content 5, http://links.lww.com/PCC/A937; and **Fig. S2**, Supplemental Digital Content 6, http://links.lww.com/PCC/A938, respectively [legend, Supplemental Digital Content 7, http://links.lww.com/PCC/A939]).

TABLE 1. Comparison of Demographic and Clinical Variables Between Inborn and Transported Infants

Variables	Inborn, *n* = 656[a]	Transported, *n* = 391[a]	Missing, *n* (%)	*p*[b]
Gestation	28.4 (26.4–29.9)	28.1 (26.4–29.7)	0	0.72
Birth weight	1,050 (810–1,285)	1,090 (860–1,300)	28 (2.7)	0.07
Male	336 (51.2)	226 (57.8)	0	0.03
Intrauterine growth restriction	81 (12.3)	39 (10)	6 (0.6)	0.24
Maternal infection risk	78 (11.9)	72 (18.4)	10 (1)	0.003
Antepartum hemorrhage	116 (17.7)	45 (11.5)	6 (0.6)	0.008
Antenatal steroid				
None/incomplete	222 (33.8)	196 (50.1)	19 (1.8)	< 0.001
Complete	423 (64.5)	187 (47.8)		
Mode of delivery				
Normal vaginal delivery	306 (47.0)	194 (49.6)	10 (1)	0.16
Emergency C-S	293 (45.0)	175 (44.8)		
Elective C-S	36 (5.5)	11 (2.8)		
Instrumental	16 (2.5)	8 (2.0)		
Intubated first 72 hr	537 (81.9)	341 (87.2)	1 (0.01)	0.01
Surfactant	535 (81.6)	342 (87.5)	0	0.009
Chest compressions	36 (5.5)	26 (6.6)	0	0.43
Adrenaline	10 (1.5)	4 (1)	0	0.5
Apgar 1 min	6 (4–8)	6 (4–8)	148 (14.4)	0.36
Apgar 5 min	9 (7–9)	8 (7–9)	161 (15.7)	0.16
Inotropes	118 (18)	113 (29)	0	< 0.001
Days to first extubation[c]	2 (1–4)	3 (1–6)	49 (4.7)	< 0.001
Mortality after day 7	39 (5.9)	27 (6.9)	0	0.52

C-S = cesarean section.
[a]Data are *n* (%) or median (interquartile range).
[b]Categorical data analyzed using χ^2 test; nonnormally distributed continuous data analyzed using Mann-Whitney *U* test.
[c]Extubation without consequent re-intubation within the following 72 hr.

TABLE 2. Unadjusted and Adjusted Odds Ratios to Show the Association of Transportation With No/Mild Intraventricular Hemorrhage and Severe Intraventricular Hemorrhage for All Infants and by Gestational Subgroups

Gestational Age Group	Outcome Comparison	OR (95% CI)	Adjusted OR (95% CI)[a]
All infants (*n* = 1,047)	No/mild IVH vs severe IVH	**1.75 (1.09–2.80)**	**1.69 (1.04–2.76)**
< 28 wk (*n* = 492)	No/mild IVH vs severe IVH	1.63 (0.94–2.82)	**1.83 (1.03–3.21)**
28–32 wk (*n* = 555)	No/mild IVH vs severe IVH	2.02 (0.77–5.35)	1.66 (0.61–4.52)

IVH = intraventricular hemorrhage, OR = odds ratio.
[a]Adjusted for gender, gestation, birth weight, mode of delivery, intrauterine growth restriction, maternal infection, antepartum hemorrhage, maternal recreational drug use, intubation at birth, surfactant administration, chest compressions at birth, delivery room adrenaline, Apgar scores 1 and 5, and neonatal ICU inotropes.
Mild, grade 1 and 2; severe, grade 3 and 4.
Bold indicates statistical significance $p < 0.05$.

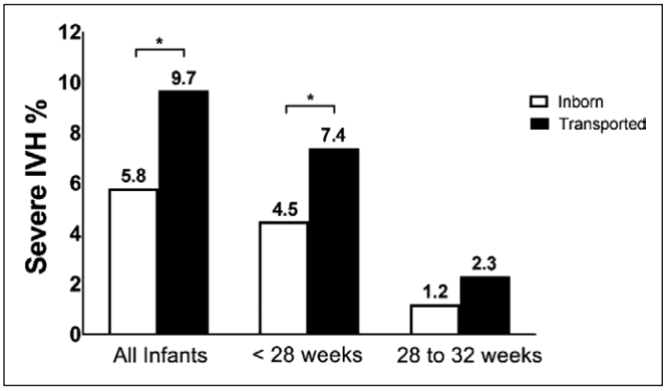

Figure 2. Comparison of proportion of severe intraventricular hemorrhage (IVH) between transported and inborn infants by gestation subgroups. *Denotes significance, $p < 0.05$. Severe IVH, grade 3 and 4 IVH.

TABLE 3. Prevalence of Intraventricular Hemorrhage on Initial and Day 7 Cranial Ultrasound

Group	CrUSS Day 1–3[a], *n* (%)	CrUSS Day 7, *n* (%)	Change (%)
Inborn			
None	426 (65)	391 (59.7)	–5.3
Mild IVH	200 (30.5)	226 (34.5)	4.0
Severe IVH	29 (4.4)	38 (5.8)	1.4
Unknown	1	1	
Outborn			
None	250 (80.6)	226 (57.8)	–22.8
Mild IVH	53 (17.1)	127 (32.5)	15.4
Severe IVH	7 (2.3)	38 (9.7)	7.4
Unknown	81[b]	0	

CrUSS = cranial ultrasound scan, IVH = intraventricular hemorrhage.
[a]CrUSS obtained prior to transport in outborn group.
[b]CrUSS obtained after transportation.

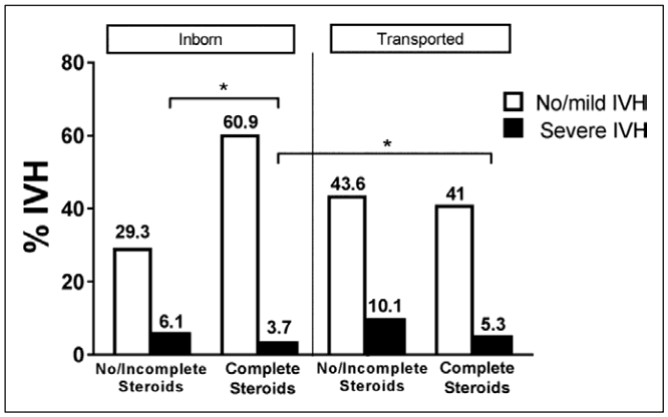

Figure 3. Comparison of proportion of no/mild intraventricular hemorrhage (IVH) and severe IVH in inborn and transported infants less than 28 wk gestation, subgrouped by antenatal steroid course. *Denotes significance, $p < 0.05$. No/mild, none/grade 1 and 2; severe, grade 3 and 4.

DISCUSSION

This study aimed to evaluate the association between early interhospital transport of preterm infants and severe IVH in the first week of life. We found transported infants, particularly those less than 28 weeks GA, were significantly more likely to develop severe IVH compared with inborns and this association remained following adjustment for major confounding factors. ANSs reduce the risk of IVH (5) but previous preterm transport studies have not been able to include these in their modeling (8, 14, 15). This UK regional network transport study is one of the largest to date and demonstrates a 67% reduction in severe IVH for all preterm infants following a full course of ANS as expected (19). However, infants less than 28 weeks GA undergoing early interhospital transport were still more likely to develop a severe IVH despite a full course of ANS.

This is the first study to demonstrate the association between early transport of preterm infants and the risk of significant brain injury in the first week of life. Our results are consistent with previous studies (2, 3, 8) demonstrating an increased risk of severe IVH at discharge in preterm infants transported early in life, however, none of these studies prove causation. The perinatal period is a high-risk window for the development of IVH, and our study raises the possibility that the postnatal transport pathway itself may contribute to the increased prevalence observed as we were able to adjust for many of the known obstetric and early neonatal risks. Our findings are similar to a recent Canadian study of tertiary center inborn and outborn infants, less than 29 weeks gestation, demonstrating significantly greater mortality, severe IVH and poorer long-term neurodevelopmental outcomes in outborn infants (12). Their study also controlled for a number of perinatal factors but did not report CrUSS findings in the first week of life, which may explain why the prevalence of severe IVH was higher than we report in the first week of life. Additional explanations for the difference could include later neonatal factors, such as sepsis, impacting on the progression of mild IVH into more severe IVH or the transport distances between Canadian centers are far greater than in the United Kingdom resulting in longer exposure to the noxious effects of transport including air transfer (20).

The mechanism for the association between IVH and transportation is not yet fully understood but is likely to be multifactorial due to suboptimal ventilation (8), temperature instability (21), and the ambulance environment. The preterm infant is exposed to many noxious agents including noise, handling, and vibration, which increase discomfort (22). Excess noise adversely impacts cardiorespiratory stability and significantly decreases cerebral oxygen saturations relative to baseline which could contribute to adverse neurologic outcomes through resultant changes in cerebral vasculature and blood flow (4, 23). Vibration is known to result in cerebral capillary wall thickening, constriction, and destruction as well as induce neuronal injury in animal models (24, 25). During neonatal ambulance transfer, the newborn's head is exposed to excessive vibration far in excess of that deemed safe and known to cause illness in well adults (26). Combined with our data,

TABLE 4. Evaluation for the Association of Transport With Severe Intraventricular Hemorrhage by Antenatal Steroids Status and Gestational Subgroup

Gestational Age Group	Outcome Variable	OR (95% CI)	Adjusted OR (95% CI)[a]
All infants (*n* = 1,047)	NS and severe IVH	1.45 (0.80–2.65)	1.44 (0.76–2.72)
	CS and severe IVH	1.70 (0.76–3.78)	1.91 (0.82–4.41)
< 28 wk (*n* = 492)	NS and severe IVH	1.12 (0.55–2.29)	1.35 (0.64–2.84)
	CS and severe IVH	2.13 (0.87–5.27)	**2.84 (1.08–7.47)**
28–32 wk (*n* = 555)	NS and severe IVH	2.57 (0.75–9.0)	3.19 (0.68–15.01)
	CS and severe IVH	0.57 (0.06–5.21)	0.47 (0.05–4.83)

CS = complete steroids, IVH = intraventricular hemorrhage, NS = no/incomplete steroids, OR = odds ratio.

[a]Adjusted for gender, gestation, birth weight, mode of delivery, intrauterine growth restriction, maternal infection, antepartum hemorrhage, maternal recreational drug use, intubation at birth, surfactant administration, chest compressions at birth, delivery room adrenaline, Apgar scores 1 and 5, and neonatal ICU inotropes.

Severe IVH, grade 3 and 4 IVH.

Bold denotes significance $p < 0.05$.

these studies potentially implicate excess vibration and noise as an additional risk factor for the development of IVH.

In this study, the higher prevalence of severe IVH in transported infants less than 28 weeks GA (4.9% vs 2.7%) for no or an incomplete ANS course lacked statistical significance. This could be as a consequence of the smaller overall numbers and a lower event rate of severe IVH in this group compared with those with a full ANS course.

This study has several strengths compared with previous studies (8, 13–15). Our study included only infants who were less than 32 weeks gestation, transferred within the first 72 hours and had a standardized day 7 CrUSS. These infants are those most at risk of IVH as the fragile germinal matrix is most prominent in this GA group (4). Using early CrUSS for outcome analysis allows for the potential inflammatory process associated with the transportation process to evolve but minimizes the influence of exposure to other postnatal events with later brain injury. Multivariate logistic regression analyses allowed adjustment for major risk factors for IVH and any group imbalance. However, we acknowledge some residual confounding effect may remain due to variable data entry error or imprecision, such as inotrope use to represent hypotension rather than actual values.

The main limitation of studies comparing inborn to transported infants is selection bias, as critically ill infants who are not stable enough to survive transport are often excluded. Our study highlights this, as inborn infants who died before day 7 were more likely to die within the first 2 days of life than transported infants. These infants were extremely premature, died shortly after delivery, and if delivered in a nontertiary center may not have survived transportation. We aimed to minimize this bias by excluding infants who died in the first week of life as many died from causes related to extreme prematurity and respiratory conditions rather than IVH. However, we cannot exclude all bias due to the increased level of care offered in tertiary centers as inborn infants, who would otherwise be too unstable for transfer if born elsewhere, have an increased chance of survival and are more likely to develop severe IVH (1) although both groups analyzed were well matched for gestation and birth weight.

A further limitation of this study was the exact timing of occurrence of IVH could not be established, although the inclusion of all infants who survived to day 7 CrUSS decreased the chance of selection bias. A prospective study could obtain a pre-transfer CrUSS to aid with interpretation in this setting. Although we used a standardized CrUSS protocol, inter-assessor interpretation could introduce differences in grading of IVH. Pragmatically, this is what happens in clinical practice, but we did try to mitigate this by subgrouping into mild (grade 1 or 2) or severe IVH (grade 3 or 4).

The limitations of retrospective cohort studies also make it difficult to account for evolving practices over the timeframe of the study. For example, the gradual introduction of magnesium sulphate, known to reduce the prevalence of cerebral palsy (27), could not be assessed although this is unlikely to affect the prevalence of early IVH.

CONCLUSIONS

Our U.K. Trent perinatal network study highlights that, despite the increased survival observed with centralized neonatal care (28, 29), the early postnatal transport of extremely preterm infants is associated with an increased risk of severe IVH in the first week of life. This risk is not completely mitigated by the known neuroprotective effects of ANS, although receiving a full course of ANS was beneficial over having either no or an incomplete course. Women presenting with threatened preterm delivery should be given prompt ANS (30) and ideally transferred in-utero to an appropriate center, a measure that could be used as a quality benchmark for perinatal network delivery of care.

With the centralization of neonatal intensive care, we have seen investment in postnatal transport services. However, the in-utero transfer process remains a time-consuming process for healthcare staff potentially resulting in missed transfer opportunities (31). Development of a coordinated, in-utero transfer service, with both obstetric and neonatal services,

could result in not only better service provision and allocation of facilities based on clinical needs but could help reduce adverse outcomes associated with postnatal transfer. However, in-utero transfer is not always achievable, therefore the associated risks of postnatal transfer need to be explored and addressed where possible to improve the comfort of the infant and minimize any risk of neurologic insult. This could include reducing noxious environmental stimuli such as noise and vibration, improving monitoring and ventilation as well as considering the timing of postnatal transfer with the ultimate goal to reduce the significant long-term risk of neurodisability.

ACKNOWLEDGMENTS

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Supplementary Online Content

Risk of Severe Intraventricular Hemorrhage in the First Week of Life in Preterm infants Transported before 72 hours of Age

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Table S1. Day of death of infants who died prior to day 7

	Day of Death (days)	
	Early (0-2)	**Late (3-7)**
Inborn (n=54)	35	19
Transported (n=11)	4	7

Table S2. Major contributing factors to the cause of death as documented on the death certificate for infants who died prior to day 7. Gestation and birth weight are median and inter-quartile range

Group	Gestation (weeks)	Birth weight (grams)	Prematurity	Respiratory	Sepsis	IVH	Congenital	Other
Inborn (n=54)	26 (24-28)	800 (620-1160)	47	30	22	6	12	23
Transported (n=11)	24 (23-26)	685 (630-930)	9	7	3	4	0	4

IVH, Intraventricular Haemorrhage

Table S3. Grades of intraventricular haemorrhage in infants who died prior to day 7

IVH Grade	Inborn (n=54)	Transported (n=11)
No scan	9 (16.7%)	0
No or mild IVH (grade 1 & 2)	38 (70.4%)	8 (72.7%)
Severe IVH (grade 3 & 4)	7 (13%)	3 (27.3%)

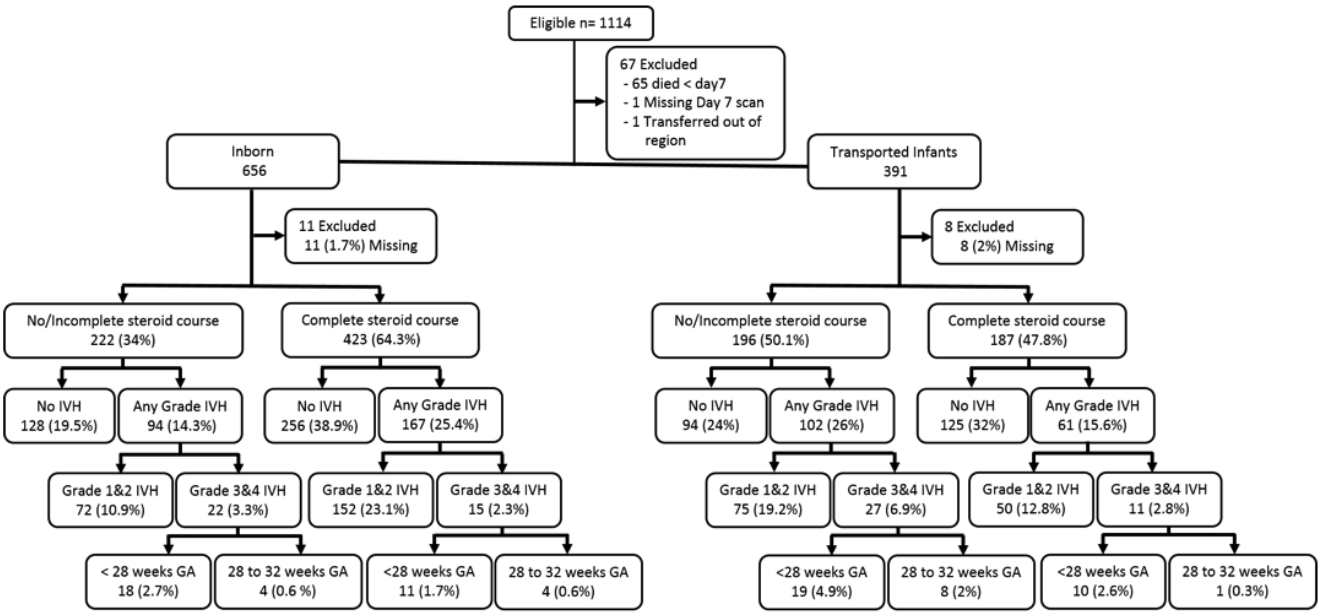
IVH, Intraventricular Haemorrhage

Table S4. Incidence of intraventricular haemorrhage in relation to time of transfer

	All infants (n= 391)		< 28 week infants (n=190)		28 to 32 week infants (n=201)	
Time of transfer (hrs)	**None/mild IVH**	**Severe IVH**	**None/mild IVH**	**Severe IVH**	**None/mild IVH**	**Severe IVH**
<24	161 (41.2%)	17 (4.3%)	73 (38.4%)	12 (6.3%)	88 (43.8%)	5 (2.5%)
24 to 48	155 (39.6%)	18 (4.6%)	78 (41.1%)	15 (7.9%)	77 (38.3%)	3 (1.5%)
48 to 72	37 (9.5%)	3 (0.8%)	10 (5.3%)	2 (1.1%)	27 (13.4%)	1 (4.5%)

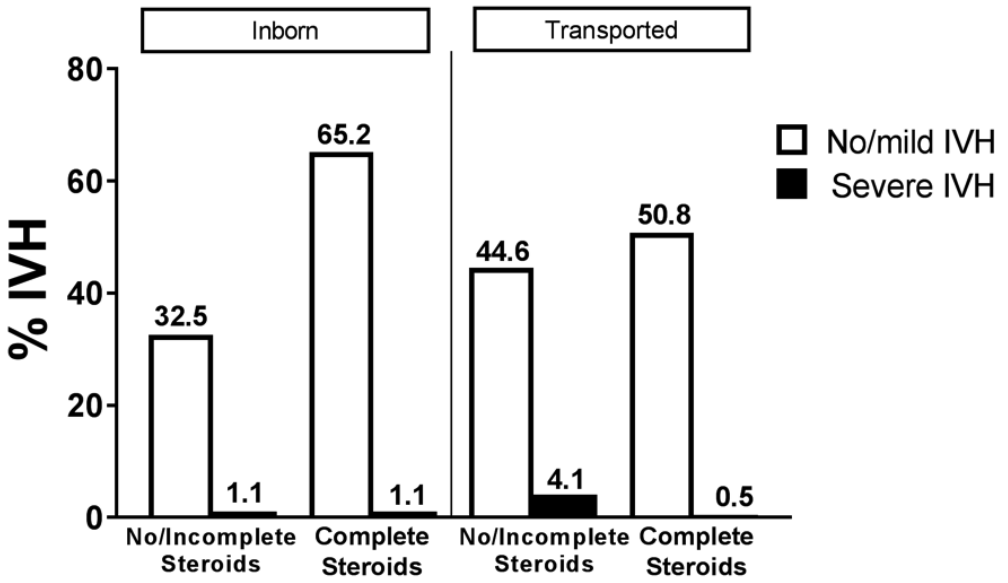
IVH, Intraventricular Haemorrhage

Figure S1. Number of infants who received either no/incomplete or complete course of maternal antenatal steroids and developed any intraventricular haemorrhage or severe intraventricular haemorrhage for both transported and inborn groups



IVH, Intraventricular Haemorrhage; GA, Gestational Age

Figure S2. Comparison of proportion of No/Mild intraventricular haemorrhage and severe intraventricular haemorrhage in inborn and transported Infants 28 to 32 weeks' gestation, sub-grouped by antenatal steroid course



IVH, Intraventricular Haemorrhage; No/mild, none/grade 1 & 2; Severe, grade 3 & 4.

Paper 3

Shipley L, Bosman C and Sharkey D. Quantifying the impact of centralised neonatal care on the family: A national population study

(Paper is currently submitted and under review by a peer-reviewed journal)

Quantifying the impact of centralised neonatal care on the family: A national population study

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Keywords: Centralisation, parental stress, preterm, transport, maternal-infant separation, NNRD

Objective

Parents of preterm infants are at greater risk of mental health problems. We aimed to quantify the impact of centralised neonatal intensive care on family stress.

Design

Retrospective cohort study using national data.

Setting

UK neonatal units.

Patients

14719 infants 23 to 27^{+6} weeks' gestation.

Main Outcomes

Primary outcome was the length of time cared for away from the maternal booking hospital (BH). Secondary outcomes included maternal-infant separation, distance from family residence and infant deaths away from BH. Temporal changes were compared between epoch 1 (2011-2013) and 2 (2014-2016).

Results

8622 (59%) infants were cared for away from their BH for a median of 39 days (IQR 15-69) with almost 30% spending >60 days away. 2803 (19%) infants underwent transfer away from their BH on day 1 of life. Infants were cared for away from their BH a median of 2 episodes (range 1-12). Median return road distances for parents to the BH was 13km (IQR 8-26) but increased to 74km (IQR 64-148) for those cared for in the non-BH. 3013 infants died during the study period, 1447 (48%) away from their BH.

Conclusion

Centralised neonatal intensive care introduces previously unquantified stresses on

parents already at significant risk of mental health problems. These include prolonged periods away from their home, significant travelling distances and half of infant deaths away from home. Parental support, through clinical psychologists, accommodation and travel costs, along with facilitation of in-utero transfers to prevent additional infant morbidity, could help minimise the stress on these vulnerable families.

Introduction

Each year in the UK there are 36,000 preterm infants admitted to neonatal units (1). Centralisation of neonatal intensive care has led to a reduction in mortality of extremely preterm infants (2-6) but increases the number of infants requiring care away from their booking hospital (BH). Ideally, in-utero transfer (IUT) of the high-risk fetus, particularly those likely to be extremely preterm, to a level 3 centre with a neonatal intensive care unit (NICU) improves mortality and minimises the risk of severe brain injury associated with postnatal transfer (PNT) (7-10).

Parents experiencing preterm birth have many unexpected stresses which can predispose them to an increased risk of anxiety, depression, poor bonding and family dysfunction that can persist for many years (11-13). Additional stress can occur if their baby needs care away from their BH as the parents need to travel long distances and reside away from their other children and support network for prolonged periods(14). These aspects are particularly important if a baby dies away from their BH as the parents may be more isolated without their family support nearby and can make bereavement follow-up more difficult. Other pressures, such as the financial implications of regular travel, car parking and sustenance can create further parental stress(15). A recent report found that only 5 of 29 NICUs surveyed had sufficient parent accommodation to meet national guidance, resulting in some parents needing alternative residence or frequent trips from their home(14).

When IUT isn't possible, the resultant early PNT can result in maternal-infant separation until follow on maternal transfer can safely occur. Furthermore, PNT of

preterm infants is associated with a greater risk of severe brain injury, which could further compound the stress these parents are exposed too (7-10).

Many of these issues could become more prevalent with a rising preterm birth rate with better survival, lowering of the boundaries of viability (16), and staff shortages (17). These may require more infants to be transferred and increase demand on an already stretched cot capacity (18). Quantifying key elements of additional stress on the family of preterm infants, associated with centralised intensive care, could help support the provision of resources to reduce these for parents at high risk of mental health problems and deliver better family integrated care.

We aimed to quantify potential stressors for families of infants, born 23 to 27^{+6} weeks gestational age (GA), cared for outside of their BH. The primary outcome was length of time cared for away from BH. Secondary outcomes included early maternal-infant separation, travelling distance from family residence, infant death away from BH and proportion of families with other children.

Methods

Study design and participants

This retrospective cohort study used prospectively collected anonymised data held in the National Neonatal Research Database (NNRD) electronic dataset from 2011 to 2016. NNRD is a Clinical Dataset (The National Neonatal Data Set) within the NHS Data Dictionary. Details of all data items are searchable(19). The NNRD is a validated database(20), which contains data on all admissions to neonatal units in the UK including demographic details, antenatal and postnatal care. The focus of this

study was on high-risk extremely preterm infants <28 weeks gestational age (GA) who were admitted to neonatal units in England, Wales and Scotland. Infants <23 weeks GA, birth weight <400 grams and those with missing hospital episodes or hospital codes were excluded. Ethical approval was given by the London-City and East Research Committee (REC: 17/LO/1822).

Outcomes

The primary outcome was the length of time the infant was cared for away from their BH until the point of discharge from the neonatal unit. Secondary outcomes were number of PNTs occurring on the first day of life (as a marker of potential maternal-infant separation), the return road distance from family residence to the non-BH providing care, the proportion of infant deaths away from their BH and the proportion of families with other children. Data from Google's online electronic route planning software was used to calculate the road distance from parental residence, defined as Lower Layer Super Output area, to the non-BH.

Statistical Analysis

Statistical analysis was performed using Stata SE (StataCorp, Version 15). Categorical data are presented as number and percentage. Continuous data are presented as median and interquartile range.

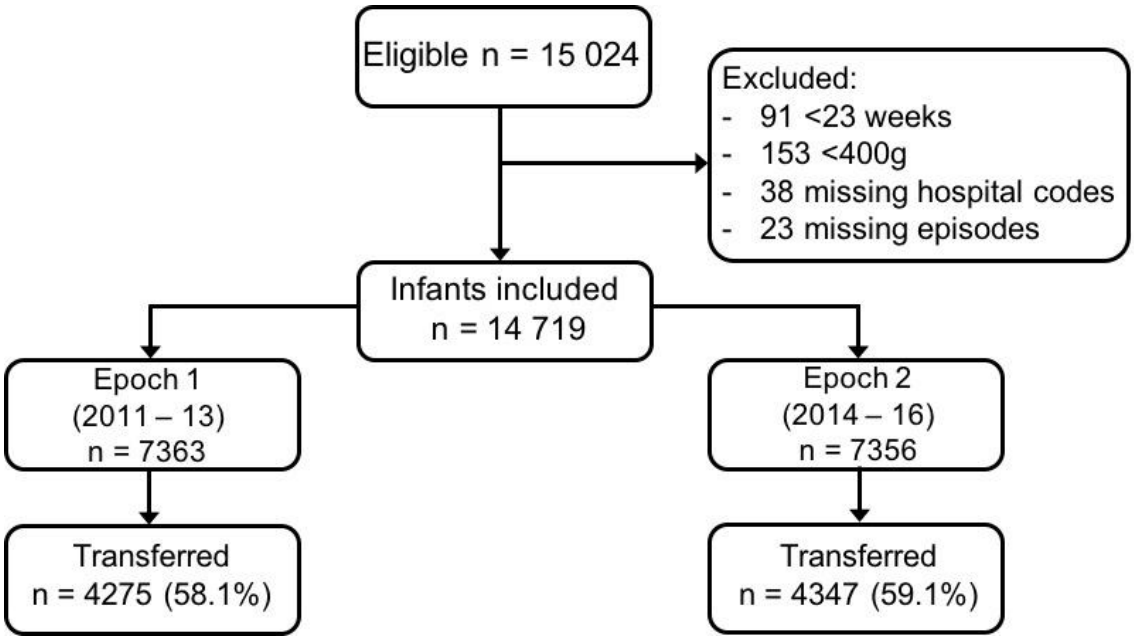
The study population was divided into two equal epochs (epoch 1: 2011 to 2013 and epoch 2: 2014 to 2016) to evaluate temporal changes. Chi squared tests for categorical data and Mann U Whitney for non-normally distributed data were used to

compare changes between epochs. Odds ratio and confidence intervals were calculated to assess temporal changes with significance set as $p<0.05$.

Results

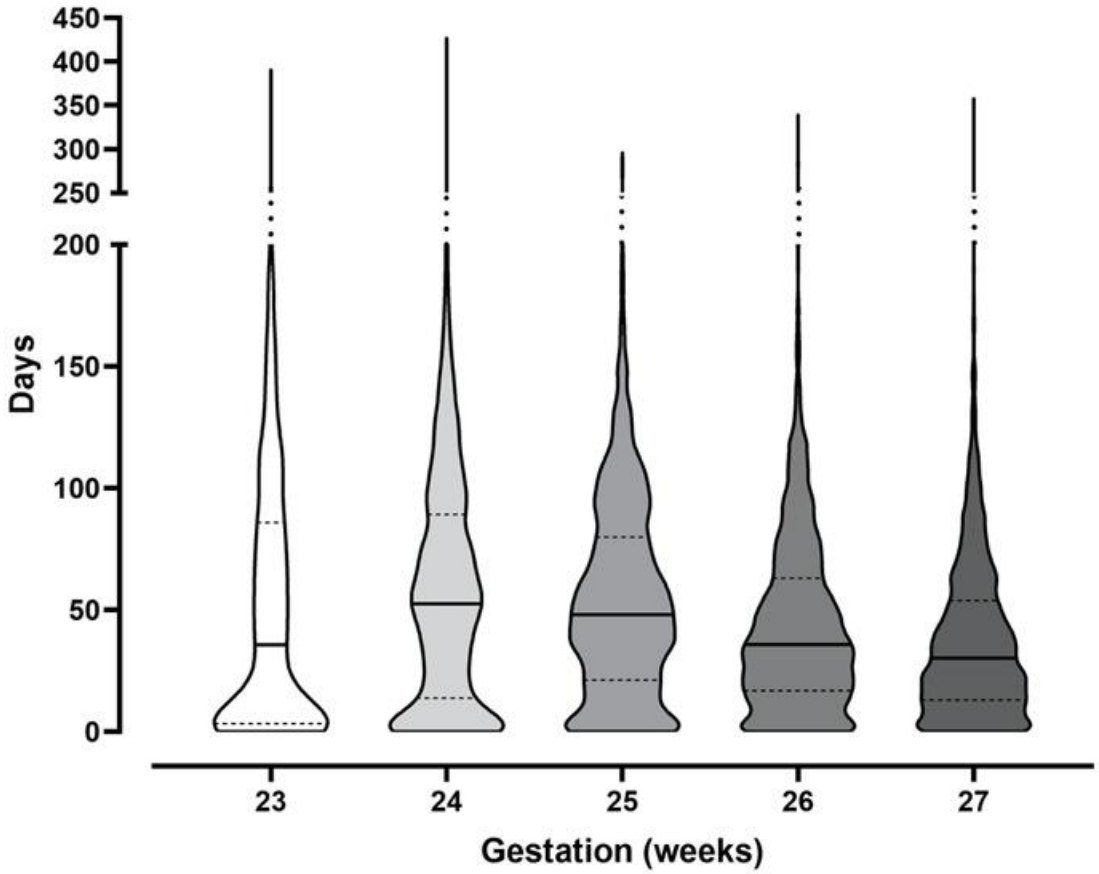
A total of 14719 infants 23 to 27^{+6} weeks were admitted to neonatal units during the study period with similar numbers between the two epochs (epoch 1 n=7363, epoch 2 n=7356, Figure 1). In the total study population, 7988 (54.3%) were male with a median gestation of 26 weeks (IQR 25-27) and birth weight 820 grams (IQR 685–970 grams).

Figure 1. Flowchart of study participants demonstrating the number of infants 23 to 27^{+6} weeks gestational age admitted to neonatal units in the UK and in each time epoch



Infants were cared for a median of 38.8 days (IQR 15- 69) away from their BH throughout their hospital admission (Figure 2). Overall, 2573 (29.8%) transferred infants spent more than 60 days being cared for away from their BH. During the study period there were a total of 408973 days where infants were cared for away from their BH, equating to approximately 28 days for every infant admitted in this population.

Figure 2. Violin plots (median and IQR) of the number of days transferred infants spent away from their booking hospital stratified by gestational age



There were 2803 (19%) infants who underwent PNT to another centre on the first day of life. Between the two epochs, PNTs on the first day of life have increased but

this was not statistically significant (epoch 1=1358, epoch 2=1445, OR 0.93, 95% CI 0.85–1.00, p=0.06).

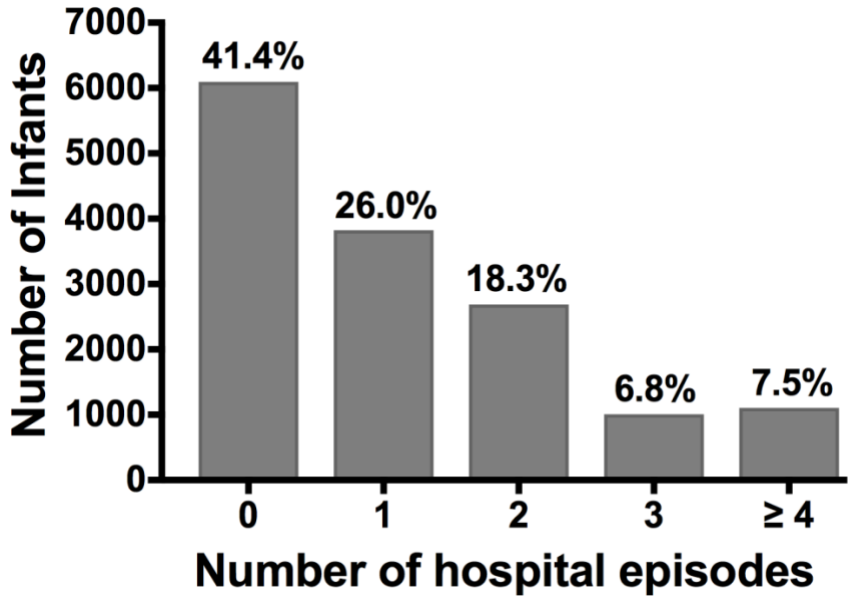
A total of 8622 (58.6%) infants underwent at least one PNT between admission and discharge (Table 1). These infants were cared for away from their BH for a median of two episodes (range 1-12) during their inpatient stay (Figure 3).

Table 1 Total number of infants 23 to 27^{+6} weeks' gestation undergoing postnatal transfer to a non-booking hospital

Gestation (weeks)	Number of Infants n (%)	Number of PNT infants n (%)	Transferred infants by GA (%)
23	1118 (7.6)	556 (6.4)	49.7
24	2474 (16.8)	1494 (17.3)	60.4
25	2868 (19.5)	1891 (21.9)	65.9
26	3684 (25.0)	2283 (26.5)	62.0
27	4575 (31.1)	2398 (27.8)	52.4

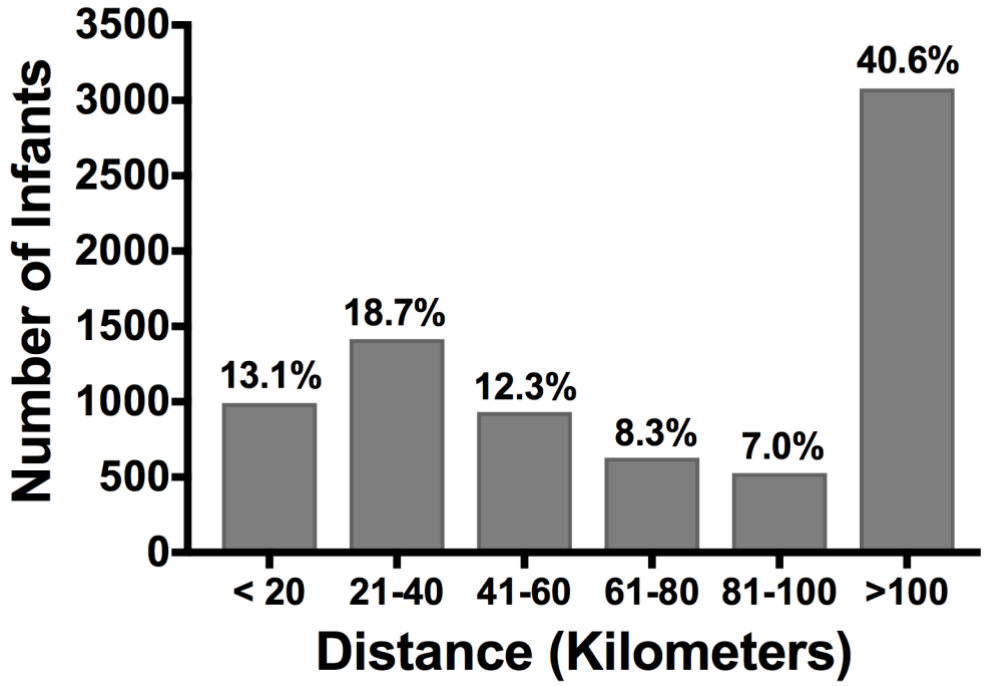
PNT, postnatal transport; GA, Gestational age

Figure 3. Number of hospital episodes cared for away from their booking hospital (n=14719) during neonatal care



The median return road distance from the maternal postcode to the BH was 13km (IQR 8-26) and for babies cared for at the non-BH this was 74km (IQR 64-148), with 40.6% of families required to travel a return journey of >100km. (Figure 4).

Figure 4. Return distance from the mother's place of residence to non-booking hospital (n=7573).



Overall, 56% (n=4802) of mothers whose babies were cared for in a non-BH had at least one previously recorded pregnancy.

A total of 3013 (20.5%) infants died during the study period of which 1447 (48%) occurred when babies were being cared for away from their BH. There was a significant reduction in deaths over time from 21.6% (n=1592) in epoch 1 to 19.3% (n=1421) in epoch 2 (OR 0.90, 0.83–0.97, p=0.01). A similar reduction was seen in deaths of babies cared for away from their BH between epoch 1 (10.7%, n=792) and epoch 2 (8.9%, n=655) (OR 0.87, 95% CI 0.75-1.00, p=0.045).

Discussion

Main Findings

Parents of preterm infants are more likely to suffer stress and mental health problems than those with well, term infants. This study aimed to quantify the burden of centralisation of neonatal intensive care as a potential additional stress on these parents. We found that 58.6% of preterm infants 23 to 27^{+6} weeks' GA were cared for away from their BH with almost a third requiring at least two or more of these episodes. Infants spent almost 39 days on average being cared for away from their BH requiring parents to travel long distances. Potential maternal-infant separation soon after birth occurred in 19% of all births following early PNT. Furthermore, we believe this is the first study to identify almost half of all infant deaths in this population occurred away from the maternal BH.

Parents of preterm infants are at increased risk of psychological distress (12,13) and the separation of a mother and her infant can increase the risk of a poor maternal mental health outcome(21). Across NICUs in England, one third of parents have no access to a trained mental health worker (17), perhaps when they are at their most vulnerable. The care of infants away from their BH could further exacerbate this risk as parents are likely to be physically located away from their support network and other children for prolonged periods of time. In the present study, care away from the BH was typically for 39 days, for 24 and 25 week GA infants this was more frequently greater than 50 days. This additional burden on parents can vary depending on the availability of hospital accommodation with many NICUs unable to provide this(14). The resultant financial cost on finding local accommodation or daily return trips to the hospital could also impact on their wellbeing.

The average return distance an infant was cared for from their parent's residence was 74km (46 miles), equating to a distance of 518 km (324 miles) a week if visiting every day, which could incur significant travel and parking costs by car. Following a caesarean section, mothers in the UK are told not to drive for 6 weeks and if the father needs to return to work these distances could be a barrier to visiting their baby.

IUT appears safer than PNT for high-risk preterm infants (22) and avoids maternal-infant separation early in life (8, 9). The high rates of PNT in our study are potentially an area that could be improved with better provision of IUT services. Worryingly, although not statistically significant, there was a 6.4% increase in PNTs over the two epochs. Fewer PNTs would reduce maternal-infant separation and decrease the risk of severe brain injury in the infant. The impact on parental wellbeing with the combination of unexpected preterm birth, early maternal-infant separation, and the devastating news of a severe intraventricular haemorrhage in the first days of life, cannot be underestimated. This combination can have implications for the long-term health of the infant, the parents and the wider family.

We found that more than half of the mothers of infants cared for away from their BH had a previous pregnancy. Although the database doesn't allow us to ascertain the number of live children, it is likely a significant number of these parents will have other children at home. This adds additional strain on the family and could negatively impact bonding as they may be unable to spend as much time with their baby as they would like. Such experiences can affect the short-term mental health of the mother and long-term outcome of the infant (23).

Over the two epochs we observed a reduction in mortality. However, an important finding of our study was that almost half of deaths occurred away from the maternal BH. For the parents this could be a barrier to receiving support without their family nearby (24). Bereaved parents may prefer follow-up with the team caring for them around the time of their infant's death but this may be more difficult if the distance is significant.

The data in this study could also be helpful when planning resources and service configuration. Recent data reported on the typical length of stay in neonatal units in the UK (25). The present study adds new information to this with quantification of length of stay away from the BH when following a centralised neonatal intensive care model. The new British Association of Perinatal Medicine Framework for extremely preterm infants adopts a more proactive approach when considering resuscitation at the extremes of prematurity including those at 22 weeks GA. This framework could result in more babies being resuscitated and admitted to NICUs. The data of our study could be a useful baseline measure of transfers and length of stay prior to any potential change in practice.

Strengths and Limitations

The main strength of our study is we used individual data from all extremely preterm infants admitted to neonatal units for England, Scotland and Wales. For the first time this has allowed a clear description of the study population, current practice of centralisation of intensive care, and provides accurate data on care away from BHs, travelling distances, early PNT and deaths.

The main limitation of this study is the use of a large prospectively collected database, resulting in missing data. We excluded infants from our study who were either missing hospital codes or admission episodes, however, these were an extremely small number of infants (0.4% of total study population). There were no differences in either GA or birth weight between these infants and those included in study analysis (data not shown). We were not able to ascertain the reason for care away from their BH for IUTs. Some of these will have been for appropriate medical reasons such as threatened preterm delivery and fetal problems. Others could for reasons such as preterm delivery whilst parents were located out of region (for example, whilst on holiday) and these could include those located the furthest away from their BH. Our study focuses on extremely preterm infants and doesn't address more mature infants who also may require care away from their BH. Finally, our aim was to quantify potential factors that, beyond extremely preterm birth, could impact on parental mental health. We were unable to establish what resources were available at each hospital to minimise this risk as it was beyond the scope of this study.

Conclusions

This study is the largest to quantify potential hidden parental stressors associated with centralised neonatal intensive care for extremely preterm infants. There is an additional burden on the families of babies not booked in centres with NICUs, with early maternal-infant separation, extended periods of time away from their home and family, and the requirement to travel significant distances. These factors can increase the financial stress on the family and reduce the support they have from their usual social networks. There could also be additional impact on the families of

those babies who die away from their BH particularly with bereavement support from both their family and those caring for them in the non-BH.

Improving the care we deliver for these families by minimising separation, supporting family integrated care and enhancing counselling and psychological services could all help minimise these additional stresses and result in better parental wellbeing and mental health. Increasing parental accommodation, financial support where needed, free hospital car parking and free or subsidised meals could all help achieve these goals. As more extremely preterm infants are surviving, we need to understand which interventions improve parental wellbeing and mental health, and how this differs for those cared for away from their home for prolonged periods.

Acknowledgements: Electronic patient data recorded at participating neonatal units that collectively form the United Kingdom Neonatal Collaborative (UKNC) are transmitted to the Neonatal Data Analysis Unit (NDAU) to form the National Neonatal Research Database (NNRD). Don Sharkey had full access to all the data in the study and takes full responsibility for the integrity of the data and accuracy of the data analysis. We are grateful to all the families that agreed to the inclusion of their baby's data in the NNRD, the health professionals who recorded data and the NDAU team.

Competing Interests: The authors have no conflict of interests to disclose. The sponsor had no involvement in the conduct of this study.

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What is already known on this topic –

- Centralised neonatal intensive care improves mortality of preterm infants but can result in transport away from their planned booking hospital
- There is currently insufficient accommodation and mental health support available for parents at many neonatal units in the UK
- Few studies have quantified potential hidden stressors related to centralised neonatal care

What this study adds-

- Almost two thirds of preterm infants are cared for away from their booking hospital during their neonatal journey
- Parents are faced with travelling long distances for prolonged periods of time and potentially away from their other children
- Almost half of infant deaths in babies born <28 weeks' gestation occur away from the maternal booking hospital and hence parental support network

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Paper 4

Shipley L, Tata L and Sharkey D. Trends in the Prevalence and Management of Hypoxic-Ischaemic Encephalopathy in the Therapeutic Hypothermia Era: A National Population Study (Draft Paper formatted for journal submission)

Trends in the Prevalence and Management of Hypoxic-Ischaemic Encephalopathy in the Therapeutic Hypothermia Era: A National Population Study

Importance: Hypoxic-ischaemic encephalopathy (HIE) remains a leading cause of mortality and neurodisability. Therapeutic hypothermia (TH) is an effective treatment in infants ≥36 weeks gestational age (GA) with moderate and severe HIE; however, there are no national population studies describing the current management of HIE following implementation of TH.

Objective: Determine the prevalence of HIE and management patterns against nationally published guidelines in England and Wales.

Design: Data were collected from the National Neonatal Research Database and Office for National Statistics (ONS) between 2011 and 2016.

Setting: National population-based cohort study of infants GA admitted to neonatal units in England and Wales

Participants: Newborns from 34 to 42 weeks GA

Main Outcome(s) and Measure(s): Patient demographics, perinatal factors, severity of HIE, mortality and management based on published guidance for TH. Temporal changes were compared across the two epochs 2011-13 and 2014-16.

Results: A total of 407,462 infants were analysed, 12,195 were diagnosed with HIE. Overall mortality for infants with HIE was 7.0% (n=851) and for those ≥36 week with moderate/severe HIE was 9.3% (n= 762). Of infants ≥36 weeks GA with HIE (n=11560), 29%

(n=3394) had mild HIE and 30% (n=1027) of these underwent TH. For late preterm (LP) infants, 34 and 35 weeks' gestation, 635 (5.2%) were diagnosed with HIE, 33% (n=210) received TH and mortality was 13.1% (n=83).

Mortality decreased significantly between Epoch 1 and 2 for moderate/severe ≥36 week infants treated with TH (15.1% vs 10.9%, OR 0.67 (95% CI 0.58-0.81), p<0.001). Between epochs, TH treatment has increased significantly in infants with mild HIE (24.7% vs 35.4%, p<0.001) and those LP (26.0% vs 39.4%, p<0.001).

Conclusions and Relevance: Mortality for infants ≥36 weeks GA with moderate/severe HIE has reduced in the TH era, potentially reflecting earlier cooling and improved care. However, TH is increasingly being used in both mild HIE and LP infants where the evidence base is lacking. The high prevalence of mild HIE and high mortality in LP infants highlights the urgent need for prospective TH studies to evaluate safety and efficacy in these populations.

Introduction

Hypoxic Ischaemic Encephalopathy (HIE) is the leading cause of mortality and neurodisability in near-term and term babies (1, 2) . It occurs in approximately 3-5 per 1000 live births with 0.5 to 1.5 per 1000 diagnosed as moderate or severe HIE (2, 3). HIE is graded as mild, moderate and severe, as described by Sarnat and Sarnat, based on the degree of neurological insult and this correlates with prognosis (4). Therapeutic hypothermia (TH) has been shown to be an effective and safe treatment for infants ≥36 weeks gestational age (GA) with moderate/severe HIE improving both mortality and 18 month survival without major neurodisability (1, 5). Following publication of the TOBY trial (6) and subsequent National Institute for Health and Care Excellence guidance in 2010 (7), TH is now the standard of care for HIE in the England and Wales. However, recent data on the current management and outcomes of infants with HIE in the TH era are lacking. The ambitious UK government target of halving birth related brain injury by 2025 requires an understanding of the current prevalence and management of HIE, allowing healthcare providers to target areas most likely to result in these improvements (8).

With the widespread adoption of TH for moderate/severe HIE in infants ≥36 weeks GA, there have been reports that more infants, namely those with mild HIE or more preterm, are also undergoing TH despite a robust evidence-base (9-13). A recent UK survey highlighted 75% of cooling centres offered TH to infants with mild HIE with a significant proportion providing sedation and modified feeding regimens (9). Although infants with mild HIE have an increased risk of mortality and adverse neurological outcomes, there is insufficient evidence to establish any significant benefits or harm with the use of TH in these infants (12, 13). For the preterm population, Azzopardi reported 3% (n=38) of UK TOBY cooling registrants were 34 or 35 weeks GA with a higher mortality rate compared to more

mature infants (30% vs 20%) (6). Rao et al (10) also found preterm infants had a higher rate of mortality and white matter injury compared to term infants. These studies were limited by the small numbers, selection bias and lack of a preterm comparator group.

The primary aim of this study was to establish the prevalence of HIE in the TH era. Secondary aims were to evaluate changes over time in the management and short-term outcomes of these infants.

Methods

Study Population

The National Neonatal Research Database (NNRD) is a Clinical Dataset (The National Neonatal Data Set), within the NHS Data Dictionary national electronic dataset, which collates prospectively collected daily clinical data from every infant admitted to a NHS neonatal unit in England and Wales. It contains data on demographic details, antenatal and postnatal care along with outcomes. Details of all data items are searchable (14). The NNRD is approved by the Standardised Committee for Care information. The data is anonymised and cleaned prior to entry into the database, screened for erroneous entries and amended by the Neonatal Data Analysis Unit, in addition to external checks by clinicians for key items. The database has been used to provide newborn brain injury data in the UK (2).

Data from the Office of National Statistics (ONS) excluding Scotland and Northern Ireland were used to calculate national prevalence rates of HIE, using live birth by GA for each year of the study period (15). Ethical approval was given by the London – City and East Research Ethics Committee (REC: 17/LO/1822).

Data Collection

Data were collected on all infants 34 to 42 weeks gestational age, who were admitted to neonatal units in England and Wales between 2011 and 2016. One level 1 unit with approximately 3000 births/year did not give consent for inclusion in the study.

Infants were identified as having HIE using the "Principle Diagnosis at Discharge" and "HIE score" data fields. Identifying NNRD data items are listed in Supplementary Table 1. Infants were allocated the worst corresponding HIE grade if differing diagnoses or HIE scores were issued across daily database entries or admission episodes if transferred to another neonatal unit. Infants identified as "birth asphyxia" or "Anoxic brain damage" were matched with the worst HIE score during admission for overall outcome grade. Infants managed with TH were identified as using the "Principle Procedures During Stay" or "Principle Diagnosis at Discharge" or "Therapeutic Hypothermia" data fields.

Infants who were greater than 5500 grams (erroneous data) and missing admission episode data were excluded from the study.

Outcome

The primary outcome was to establish the prevalence of HIE in newborn infants in the TH era. The secondary outcomes were to evaluate the management of these infants, subsequent mortality and describe any temporal changes.

Statistical Analysis

In 2011, approximately 90% of English neonatal units contributed to the NNRD and no units from Wales. From 2012 onwards, all units contributed to the database. Extrapolation methods based on actual admission numbers and number of cases from 2012 to 2016 when all units contributed to the database were used to estimate the prevalence of HIE for 2011 as previously described (2).

The population prevalence rate per 1000 for infants with HIE were calculated using NNRD data to identify cases and ONS data for live birth rates per year by GA as the denominator. The study population was equally divided into two epochs (2011 to 2013 and 2014 to 2016) to evaluate changes over time.

For subgroup analysis, infants were further divided into LP (34 to 35 weeks GA) and mild (Grade 1) HIE subgroups. Univariate analysis using chi-squared test was used to evaluate the association of death with moderate/severe HIE in infants ≥36 weeks GA and both subgroups.

Associations between demographic and clinical variables with moderate/severe HIE were assessed using chi-squared tests for categorical data and Mann U Whitney for non-normally distributed continuous data. All statistical analyses were performed using Stata SE (StataCorp, Version 15).

Results

During the study period there were 4,123,048 births in England and Wales with a GA 34 to 42 weeks. Of these, 407,707 (9.9%) infants were admitted to neonatal units and 407,462 were included in the study (Figure 1). There were 12,195 infants with any grade HIE in the study population giving a prevalence of 2.96 per 1000 livebirths and accounting for 3% of neonatal unit admissions ≥34 weeks GA. The prevalence of moderate/severe HIE in infants ≥36 weeks GA was 2.03 per 1000 (n=8166) with 1.23 per 1000 (n=4949) treated with TH (Table 1).

Figure 1: Flowchart of study participants demonstrating included/excluded infants and rate of Hypoxic Ischaemic Encephalopathy for each gestation subgroup

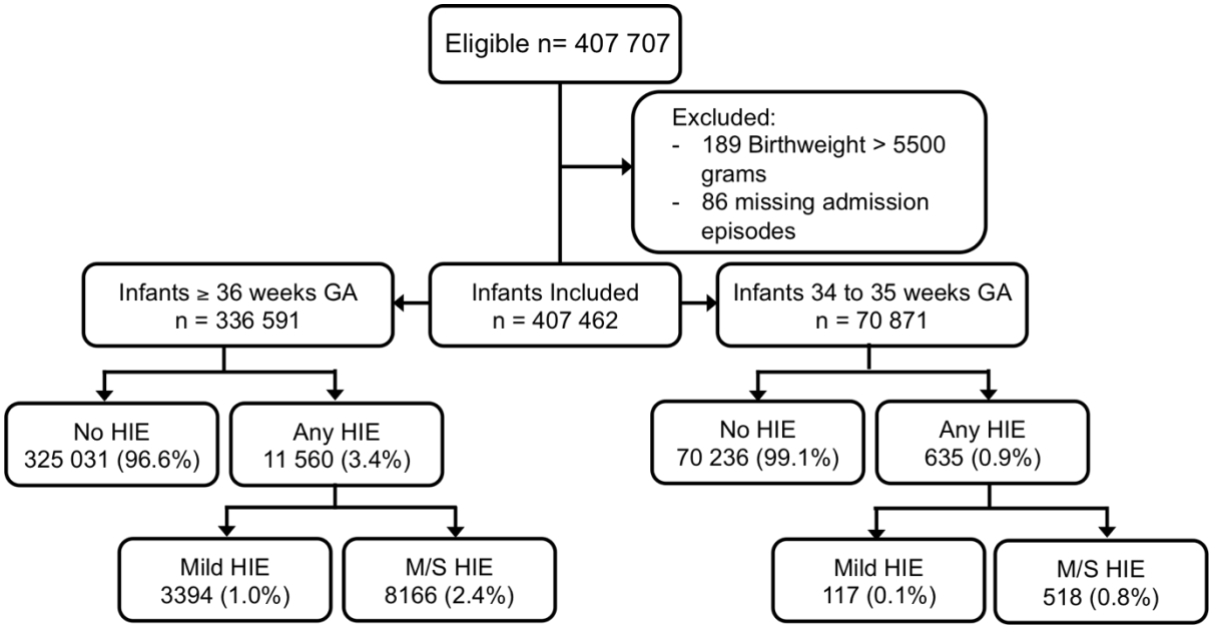


Table 1: Hypoxic Ischaemic Encephalopathy (HIE) prevalence for whole study population, infants ≥36 weeks gestational age (GA) with moderate/severe (M/S) HIE and infants ≥36 weeks gestational age with moderate/severe HIE treated with therapeutic hypothermia (TH)

Year	Any HIE (n)	Rate per 1000 live births	M/S HIE & ≥ 36 wk GA (n)	Rate per 1000 live births	M/S HIE & ≥36 wk GA & TH (n)	Rate per 1000 live births
2011[†]	2253 (2320 – 2372)	3.22 (3.32 – 3.39)	1592 (1631 – 1668)	2.33 (2.33 – 2.39)	694 (704 - 706)	1.02 (1.03)
2012	1916	2.71	1267	1.83	723	0.98
2013	1906	2.81	1213	1.83	806	1.15
2014	2135	3.17	1422	2.16	937	1.30
2015	2104	3.11	1396	2.11	928	1.30
2016	1881	2.77	1276	1.93	861	1.22
Total	12195	2.96	8166	2.03	4949	1.14
Epoch 1[†]	6075 (6142 – 6194)	2.91 (2.94 – 2.96)	4072 (4111 – 4138)	2.00 (2.01 – 2.03)	2223 (2233 - 2235)	1.09* (1.09*)
Epoch 2	6120	3.01	4094	2.07	2726	1.40*

[†] Range included to account for 2011 data not available for all hospitals (see methods)
*denotes significant change between Epoch 1 and 2, $p<0.05$

Antenatal characteristics of infants ≥36 week GA with HIE, diagnosed moderate/severe as per national guidelines (16), and the non-HIE neonatal unit (NNU) population were compared (Table 2). Infants with moderate/severe HIE were significantly more likely to be born to nulliparous mothers and have risk factors for infection. Those with moderate/severe HIE had significantly higher birth weight, including those >98th centile, acute intrapartum events, births by emergency caesarean section and the need for significant resuscitation.

Table 2. Comparison of Antenatal and Delivery Characteristics between infants ≥ 36 weeks gestational age admitted to neonatal units with and without moderate or severe hypoxic-ischaemic encephalopathy

Variable	NNU n = 325031 *	M/S HIE n = 8166 *	Missing n (%)	Odds Ratio (95% CI)	p value**
Antenatal Characteristics					
Diabetes Mellitus	5142 (1.6)	78 (1.0)	0	0.60 (0.48 – 0.75)	<0.001
Nulliparity	89876 (27.7)	3192 (39.1)	0	1.68 (1.61 – 1.76)	<0.001
Gestational diabetes	21939 (6.7)	297 (3.6)	0	0.52 (0.46 – 0.56)	<0.001
Preeclampsia	14214 (4.4)	400 (4.9)	0	1.13 (1.02 – 1.25)	0.02
Risk factors of early infection[a]	63317 (19.5)	1909 (23.4)	0	1.26 (1.20 – 1.33)	<0.001
Delivery Characteristics					
Gender	182618 (56.2)	4617 (56.5)	460 (0.1)	1.01 (0.97 – 1.06)	0.06
Gestational age (weeks)	39 (37 - 40)	40 (38 – 41)	0	1.26 (1.25 – 1.29)	<0.001
Birthweight (grams)	3270 (2805 – 3700)	3355 (2960 – 3765)	0	1.02 (1.00 – 1.02)	<0.001
>98th Centile	16301 (5.0)	464 (5.7)	0	1.14 (1.04 – 1.25)	<0.01
Intrapartum Events[b]	4744 (1.5)	870 (10.7)	0	8.05 (7.46 – 8.69)	<0.001
Significant resuscitation	7263 (2.2)	3821 (46.8)	0	38.5 (36.5 – 40.6)	<0.001

*Data are n (%) or median (interquartile range)

** Categorical data analysed using Chi squared test; Non distributed continuous data analysed using Mann-Whitney test

[a] Maternal pyrexia, Chorioamnionitis, Prolonged rupture of membranes, Urinary tract infection

[b] Cord prolapse, Shoulder dystocia, Abruption, Reduced fetal movements

[c] Chest compression, Intubation, Drugs

Mortality for infants with HIE in the total population was 7.0% (n=851) and 9.3% (n=762) for infants ≥36 weeks GA with moderate/severe HIE. Overall mortality within the study population for infants with moderate/severe HIE has not significantly decreased over time (10.3% (n= 442) vs 9.2% (n = 401), p = 0.32). However, for infants with ≥36 weeks GA moderate/severe HIE mortality decreased significantly between Epoch 1 and 2 (10.0% (n = 406) vs 8.7% (n = 356); OR 0.85 (95% CI 0.74-0.99), p= 0.04).

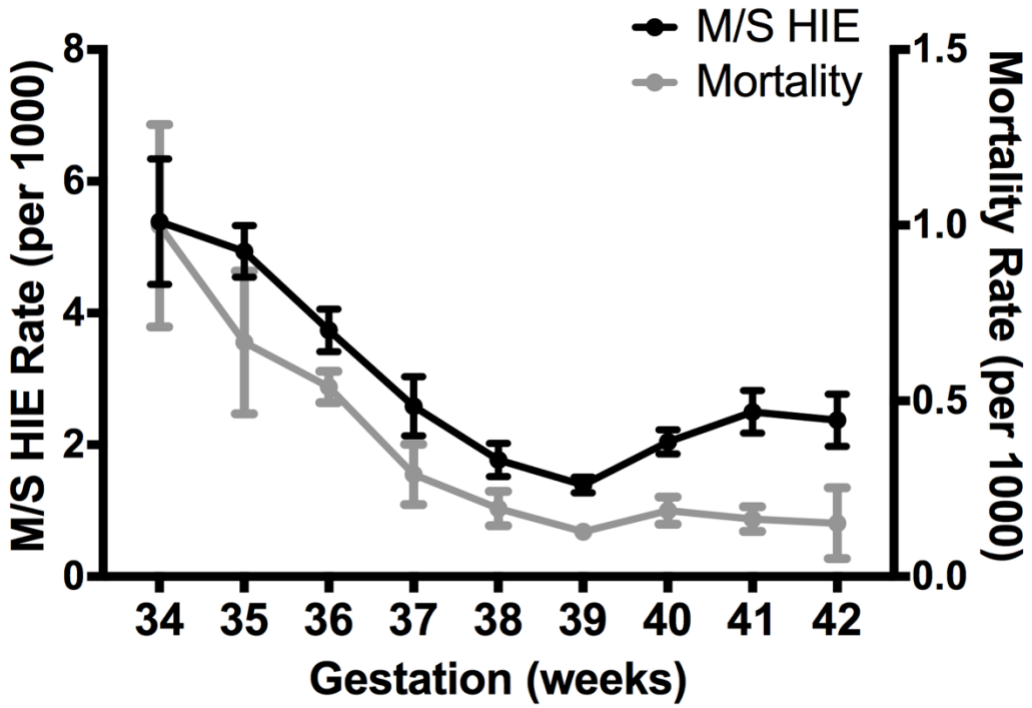
The prevalence of moderate/severe HIE in infants ≥36 weeks GA remained similar between Epoch 1 and 2 (Table 1). The use of TH in this group has significantly increased between epochs (1.09 vs 1.40 per 1000, p<0.001) associated with a significant reduction in mortality amongst treated infants (15.1% (n = 336) vs 10.9% (n = 297); OR 0.67 (0.58-0.81), p<0.001).

Late Preterm (LP) infants (34 to 35 weeks)

70,781 LP infants were admitted to neonatal units during the study period (Figure 1). In total, 635 LP infants (6.27 per 1000) were diagnosed with any grade HIE (Supplementary Table 2), 81.5% (n=518) having moderate/severe HIE. This remained stable through Epoch 1 and 2 with 6.00 per 1000 vs 6.55 per 1000 respectively (p=0.27).

210 (33%) LP infants were treated with TH, which significantly increased between Epoch 1 (n=78, 26.0%) and 2 (n=132, 39.4%, p<0.001) but mortality remained unchanged (n = 36 (12.0%) vs n = 47 (14.0%), p=0.51). The rate of moderate/severe HIE and the associated mortality were greatest in the LP population compared to those ≥36 weeks GA (Figure 2 and Supplementary Table 2). Overall, mortality in infants with moderate/severe HIE in the LP population was 15.6% (n=81) compared to those ≥36 weeks GA at 9.3% (n = 762, p=0.005).

Figure 2. Rate of Moderate or Severe Hypoxic Encephalopathy (M/S HIE) and Mortality within the study population per gestational week



Infants ≥ 36 weeks with Mild HIE

There were 3394 infants ≥36 weeks GA diagnosed with mild HIE which was similar across Epoch 1 (n=1712) and 2 (n=1682, p=0.57). Mortality was low in this population with only 6 deaths overall. The majority of infants with mild HIE were born in non-tertiary centres (n= 2097, 61.3%). 30% (n=1027) were managed with TH, 338 (32.9%) of these were transported within the first 48 hours from level 1 and 2 units into a cooling centre. Almost a quarter (n= 117) of infants who were transferred were not subsequently treated with TH. The number of transfers of infants with mild HIE was not different between Epoch 1 (n= 230) and 2 (n= 242, p= 0.48). The proportion of infants treated with TH has significantly increased from Epoch 1 (n= 423, 24.7%) and 2 (n= 604, 35.9%, p<0.001).

Discussion

This large national population based study, in the TH era, has shown the prevalence of moderate/severe HIE in infants ≥ 36 weeks GA remains similar to previous studies at 2.03 per 1000 infants. Importantly, the overall mortality rate for infants 34 to 42 weeks' GA with moderate/severe HIE has not significantly decreased over time, which reflects moderate/severe HIE remains a major cause of mortality and severe neurodisability despite recognition of risk factors (16-18) and preventative strategies (1).

Following publication of the TOBY trial in 2008 (6), our study has shown the implementation of TH as a treatment for HIE in infants ≥ 36 weeks GA has significantly increased, with a corresponding decrease in mortality. In addition, mortality in these infants treated with TH has continued to decrease since the TOBY study from 20% to 12.7% (6), which is likely to reflect a change in practice recognising and implementing treatment and improving intensive care management.

However, the number of infants diagnosed with mild HIE and treated with TH has significantly increased over time without proven benefit (12, 13) . The rate of mortality within this subgroup in our study was very low with no change over time. Management of these infants with TH risks exposing infants to unnecessary treatment with its associated morbidity, such as pain and respiratory support (20,21). TH is mainly undertaken in tertiary centres in the UK. This extra burden of treating infants with mild HIE increases the pressure on the already stretched resources, cot capacity and specialist neonatal transportation services (22) with significant associated cost to society (23). Furthermore, transportation of infants away from their birth centre could increase the risk of poor parental mental health due to several factors, such as financial burden of travel and sustenance and being located away from their support network (24-26).

To our knowledge, this is the largest study to date to evaluate the rate of HIE, use of TH and associated mortality in LP infants. Our study has shown LP infants have a higher rate of moderate/severe HIE compared to term infants. The initial TOBY study showed TH was a safe and effective treatment in infants ≥36 weeks GA (6), yet TH is increasingly been used in LP infants. The pathophysiology of brain injury in LP is likely to be different to those at term. Rao et al (10) reported on the safety of TH in infants 34 to 35 weeks GA. Although limited by small sample size (n=63), the study showed a higher incidence of white matter injury and mortality secondary to redirection of care due to the severity of the encephalopathy in LP infants. Our study also showed LP infants with moderate/severe HIE were more likely to die than infants ≥36 week GA, which highlights the need for a well-designed prospective study to evaluate the safety and efficacy in this subgroup.

Strengths and limitations

This study's main strength is the use of national data from a recognised reliable database of prospectively collected data (27). This has allowed our group to analyse a large sample size and provide an accurate estimate of the prevalence of HIE within the population. To our knowledge, this study has analysed the largest group of LP infants with HIE to date compared to previous studies (10, 11, 19, 28, 29). We acknowledge only 90% of neonatal units contributed to the NNRD in 2011, however, we were able to use a standardised approach to give a lower and upper estimate of missing data using actual data from admission and HIE rates from 2012-2016 (2). In addition, the rate of mortality in infants with HIE born in 2011 is likely to be higher than reported, however, this further would highlight the significant decrease in mortality rates between Epoch 1 and 2.

A further strength is the use of a standardised approach to diagnose HIE based on the Sarnat and TOBY criteria across the UK. Although subjective variation in grading between clinicians may occur, we have aimed to minimise this by subgrouping infants based on long term outcomes into mild (grade 1) and moderate/severe (grade 2 and 3).

The main limitation of studies using large databases are errors or imprecision in data entry. This is minimised by the neonatal data analysis unit who process and screen the data for erroneous entries, although this cannot be completely mitigated if the data is incorrect at the point of entry.

Conclusion

This study showed that the prevalence of moderate/severe HIE has remained relatively unchanged over the six year study period. The uptake of treatment with TH has significantly increased over time with a corresponding decrease in mortality. However, worryingly the use of TH outside of evidence-based practice has significantly increased. This can not only lead to substantial healthcare costs through unnecessary treatment but expose LP infants who have a higher rate of mortality to a potential risky treatment whose effect is unknown in this gestational age group. This emphasises the urgent need for a well-designed prospected study to evaluate the safety of TH in LP infants and careful re-evaluation of the management of infants with mild HIE.

Acknowledgements

Electronic patient data recorded at participating neonatal units that collectively form the United Kingdom Neonatal Collaborative (UKNC) are transmitted to the Neonatal Data Analysis Unit (NDAU) to form the National Neonatal Research Database (NNRD). DS had full access to all the data in the study and take full responsibility for the integrity of the data and accuracy of the data analysis. This research was funded by University of Nottingham.

We are grateful to all the families that agreed to the inclusion of their baby's data in the NNRD, the health professionals who recorded data and the NDAU team.

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Supplementary Online Content

Trends in the Prevalence and Management of Hypoxic-Ischaemic Encephalopathy in the Therapeutic Hypothermia Era: A National Population Study

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Supplementary table 1 Description of data fields used for determining variables and outcomes from the National Neonatal Research Database

Principle Diagnosis at Discharge database entries for HIE diagnosis and grading
Severe HIE : - HIE Grade 3 - Severe Neonatal Encephalopathy - Hypoxic ischaemic brain damage ; Severe - Severe perinatal asphyxia (with 1 minute Apgar <4) - Severe Neonatal Encephalopathy - Gr.3 - Hypoxic Ischaemic Encephalopathy (Gr 3) - Severe Neonatal Encephalopathy – Grade 3 HIE Moderate HIE: - HIE Grade 2 - Moderate Neonatal Encephalopathy - Hypoxic ischaemic brain damage ; Moderate - Moderate perinatal asphyxia (with 1 minute Apgar 4-7) - Moderate Neonatal Encephalopathy - Gr.2 - Hypoxic Ischaemic Encephalopathy (Gr 2) - Moderate Neonatal Encephalopathy - Grade 2 HIE Mild HIE: - HIE Grade 1 - Mild Neonatal Encephalopathy - Hypoxic ischaemic brain damage ; Mild - Mild perinatal asphyxia (with 1 minute Apgar >7) - Mild Neonatal Encephalopathy - Gr.1 - Hypoxic Ischaemic Encephalopathy (Gr 1) - Mild Neonatal Encephalopathy - Grade 1 HIE - Very mild perinatal asphyxia - clinically normal by 24 hours Unspecified - Birth Asphyxia - Anoxic Brain Damage Therapeutic Hypothermia - Therapeutic Hypothermia - Therapeutic Hypothermia (whole body cooling) - Hypothermia Therapeutic
Principle procedures during stay entries for identification of Therapeutic Hypothermia
Therapeutic Hypothermia - Therapeutic Hypothermia

- Therapeutic Hypothermia (whole body cooling)
- Hypothermia Therapeutic

Demographic and Clinical Variables

- Gender
- Birthweight
- >98th Centile determined from data field "Birthweight"
- Gestation in weeks
- Year of Birth
- Number of previous pregnancies
- Diabetes Mellitus determined from data field "Medical problems prior to pregnancy"
- Maternal Infection determined from data fields "Maternal pyrexia in labour" and "Problems during pregnancy with mother" (maternal UTI /chorioamnionitis/prolonged rupture of membranes)
- Pre-eclampsia determined from data field problems during pregnancy with mother
- Gestational Diabetes determined from data field "Problems during pregnancy with mother"
- Acute intrapartum events determined from data fields "Problems (obstetric) during pregnancy with mother" (Reduced fetal movements/placental abruption/cord problems/shoulder dystocia) and "Principle diagnosis on discharge"
- Significant resuscitation determined from data field "Methods of resuscitation" (cardiac compressions/intubation/adrenaline/other drugs)

Outcome Variable

- Death determined from Destination at Discharge data field. If an infant was discharged "home", "ward" or "Foster Care" the infants was coded as surviving to discharge.

Supplementary table 2 Prevalence rates for the whole study population by gestational age for any grade of Hypoxic Ischaemic Encephalopathy (HIE), moderate/severe HIE and mortality with moderate/severe HIE

Gestational Age	Any grade HIE n = 12195	Rate per 1000 live births	M/S HIE n = 8684	Rate per 1000 live births	Died & M/S HIE n = 843	Rate per 1000 live births
34	255	6.22	221	5.39	41	1.00
35	380	6.31	297	4.93	40	0.66
36	615	5.04	468	3.84	66	0.54
37	977	3.50	719	2.58	81	0.29
38	1344	2.38	996	1.77	109	0.19
39	1989	1.97	1400	1.39	129	0.13
40	3319	2.94	2313	2.05	212	0.19
41	2837	3.63	1946	2.49	144	0.18
42	479	3.49	324	2.36	21	0.15
Total						
≥36 wks	11560	3.03	8166	2.03	762	0.19
34 to 35 wks	635	6.27	518	5.12	81	0.80

GA, Gestational Age; HIE, Hypoxic Ischaemic Encephalopathy; M/S, moderate/severe

Paper 5

Shipley L, Mistry A and Sharkey D. Association of birth in centres without active therapeutic hypothermia and seizure free survival following neonatal hypoxic ischaemic encephalopathy: A nationwide study

(Paper is currently submitted and under review by a peer-reviewed journal)

Association of birth in centres without active therapeutic hypothermia and seizure-free survival following neonatal hypoxic ischaemic encephalopathy: A nationwide study

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Word Count: 2933

Key Points

Question: What is the association between seizure-free survival in infants with hypoxic-ischaemic encephalopathy born in centres without active therapeutic hypothermia?

Findings: In this nationwide cohort study of 5059 newborn infants with hypoxic-ischaemic encephalopathy, 47% of births were in centres without access to active therapeutic hypothermia and this was associated with reduced seizure-free survival after controlling for key risk factors and level of birth hospital.

Meaning: Infants with hypoxic-ischaemic encephalopathy born in centres without active therapeutic hypothermia are less likely to survive without seizures which could adversely affect their long-term neurodevelopmental outcome.

Importance: Treatment of hypoxic-ischaemic encephalopathy (HIE) with therapeutic hypothermia (TH), commenced within 6 hours of age, improves survival and reduces disability. Infants with seizures related to HIE have worse outcomes. In many developed settings, TH is provided in centralised cooling centres (CC) so requiring the transfer of infants born in non-cooling centres (non-CC). It is unclear if birth in a non-CC impacts on short-term outcomes.

Objectives: To determine if birth in a centre without active TH is associated with seizure-free survival.

Design, setting and participants: This retrospective population-based cohort study used nationally collected data from the UK National Neonatal Research Database from 2011 to 2016. Newborn infants ≥36 weeks gestational age with moderate or severe HIE were included if they received TH or died during their neonatal admission. Infants were propensity score-matched on predefined perinatal variables to form well-balanced groups, and subsequently compared based on birth hospital provision of active TH.

Exposure: Immediate access to active TH at birth centre.

Main Outcomes and Measures: Primary outcome was survival without seizures. Secondary outcomes were seizures, mortality and temperature on arrival at the CC following transfer from a non-CC.

Results: A total of 5059 infants were identified; 2364 (46.7%) were born in a non-CC. Following matching, compared with those born in a non-CC, birth in a CC was significantly associated with improved survival without seizures (35.1% vs 31.8%;

OR 1.15, 95% CI 1.02-1.31; p=0.02), fewer seizures (60.7% vs 64.6%; OR 0.84, 95% CI 0.75-0.95, p=0.007) but similar mortality (15.8% vs 14.4%; OR 1.11, 95% CI 0.93-1.31, p=0.20). For births in level 2 centres only, seizure-free survival remained significantly greater for those born in CCs (38.8% vs 32.3%; OR 1.33, 1.06-1.65, P=0.01) with fewer requiring anticonvulsants. Following transfer from a non-CC (n=2027), only 259 infants (12.7%) arrived at the CC with a temperature in the therapeutic range within 6 hours of birth.

Conclusions and Relevance:

Almost half of UK infants with HIE were born in centres without TH and this was associated with delay in optimal treatment and lower seizure-free survival. Provision of active TH in every birthing hospital warrants further investigation.

Introduction

Moderate or severe hypoxic ischaemic encephalopathy (HIE) is the leading cause of mortality and neurodisability in infants ≥36 weeks gestational age (GA) affecting 0.5-1.5/1000 births (1). In this population, active therapeutic hypothermia (TH) commenced within the first 6 hours post insult is safe (2, 3) and significantly improves survival and neurodisability (4-7). Active TH for moderate/severe HIE has been successfully established in the UK (8) within specialist cooling centres (CCs). Infants born in non-cooling centres (non-CCs) are usually passively cooled and transferred via designated transport teams to regional CCs for active TH. Although many neonatal transport teams use active TH during transportation, improving therapeutic temperature on arrival at the CC (9), their availability, despatch time and travelling distance can cause delays achieving target temperature for babies in non-CCs. Any delay, particularly beyond 6 hours of age, could reduce the benefits of TH. Furthermore, studies have reported early TH (<3 hours of age) is associated with better neurodevelopmental outcomes (10, 11), although this has not been universally demonstrated (12,13).

In addition to the severity of HIE, seizures are also increasingly recognised to impact on long-term neurodevelopment. Both animal (14, 15) and human studies (16-19) of HIE demonstrate that increasing seizure burden is associated with more severe brain injury and adverse neurodevelopmental outcomes, and this may be independent of HIE severity (16, 20). Furthermore, studies have suggested that TH for HIE suppresses and reduces the overall burden of seizures (14, 21-23) leading some to conclude that seizures add further insult to the underlying brain injury and is potentially one of the mechanisms by which TH protects the brain (23, 24). Seizures

associated with HIE can also have a significant impact on the parents mental health with over half having symptoms of anxiety and depression (25).

With the centralisation of CCs, there is requirement to transfer many infants for TH soon after their insult and potentially during a period of increased susceptibility to secondary brain injury. Postnatal transport of very preterm infants during early life is associated with a greater risk of brain injury (26, 27), potentially as a result of the adverse ambulance environment. It is unclear if the transport of newborns with HIE impacts on their outcomes as studies have been small and contradictory (28, 29). No study has explored the impact of a nationwide approach to centralised TH for infants with HIE and the impact on outcomes.

The primary aim of this study was to evaluate the relationship between birth in a non-CC and survival without seizures in infants ≥36 weeks GA with moderate/severe HIE. The secondary aims were to compare mortality, seizures only and explore admission temperatures for infants transported to a CC. Performing a randomised clinical trial to establish this would be ethically challenging and would require a significant number of participants over a number of years. We therefore utilised propensity score-matching to balance early life confounders in infants born in non-CCs and CCs using national, routinely collected clinical data.

Methods

Study Design and Data Sources

We performed a retrospective cohort study using data from the UK National Neonatal Research Database (NNRD). This is a validated national electronic database (30) containing prospectively collected demographic and clinical data including outcomes on all infants admitted to over 180 UK neonatal units. The NNRD uses a designated approved dataset (National Neonatal Data Set) within the NHS Data Dictionary, details of all data items are searchable (31). The NNRD is approved by the Standardised Committee for Care Information. The data is anonymised and cleaned prior to entry into the database; erroneous entries are queried and amended by the Neonatal Data Analysis Unit.

Data were collected on all infants 36 to 42 weeks' GA, admitted to neonatal units in the UK between 2011 and 2016, with a diagnosis of moderate/severe HIE and underwent TH. Infants who had a diagnosis of moderate/severe HIE and subsequently died within the first 48 hours of life, but did not undergo TH, were also included within the study population. In 2011, 90% of neonatal units contributed to the UK dataset with almost 100% contributing from 2012. Infants with a diagnosis of HIE managed with TH or died, were identified using the data fields from the NNRD (Supplementary Table 1). The most severe grade of HIE identified across daily database entries, or admission episodes if transferred to another neonatal unit, was allocated for each infant. Infants identified as "birth asphyxia" or "anoxic brain damage" were matched with the worst HIE score during admission for overall outcome grade. Infants were excluded if their birth weight was >2 standard deviations above the 99.6th weight centile (erroneous data), those born in private

hospitals or with missing birth hospital codes. The identification of CCs, and their date of commencement of TH, was cross checked using the TOBY trial register (4) and via direct communication with centres.

Ethical approval was given by the London – City and East Research Ethics Committee (REC: 17/LO/1822). The study protocol was sent to all participating UK neonatal units prior to data extraction.

Study Measures

The primary outcome was survival without seizures to discharge in infants ≥36 weeks GA with moderate/severe HIE. The secondary outcomes were mortality, seizures alone, and anticonvulsant use during the same period. We also evaluated the admission temperature from time of birth on arrival at a CC following transport from a non-CC.

Statistical Analysis

The prevalence of background variables between infants born in non-CCs and CCs were compared using chi-squared test for categorical data and Mann-Whitney U test for non-normally distributed data.

Subsequently, propensity score-matching was used to provide balanced groups of infants matched on demographic and clinical covariates within treatment groups (32, 33). Propensity score analysis entailed fitting a logistic regression model using a priori variables followed by a stepwise process and evaluation of potential interactions to determine covariates to include in the propensity score model

(Supplementary eMethods). Propensities were trimmed to exclude extreme values and minimise potential residual bias. The propensity score was used to match infants in the CC group to infants in the non-CC group using the nearest neighbour 1:1 matching algorithm with caliper width set to 0.05.

Balancing of the groups post-matching was evaluated by the standardised difference and mean bias between control and treatment groups (Supplementary eMethods, Supplementary Figure 1 and Table 1) (34). A covariate was considered balanced if the standardised differences were between -0.2 and 0.2 and standardised mean bias was <5% (34, 35). Matched groups of infants from non-CC and CC were identified and the effect of birth in a CC on outcomes was described using odd ratios (36). Data were statistically analysed using Stata SE (StataCorp, Version 16).

Sensitivity Analyses

We performed two further paired, matched analyses between infants born in level 2 non-CCs and CCs and between infants born in a non-CC and CC but excluding those born either at home or in midwife led units. The propensity matching methodology was undertaken as described above and in the supplementary data (Supplementary eMethods, Supplementary Figure 2 and 3). These additional analyses were performed to evaluate the reliability of our findings by minimising potential bias through differences in intrapartum and early neonatal care.

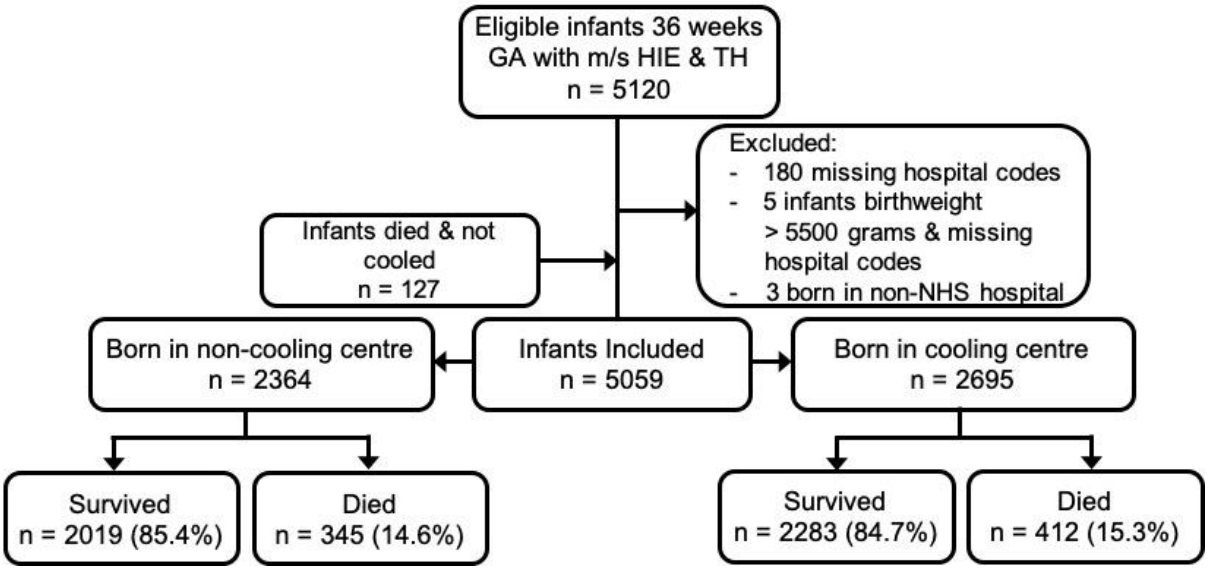
Results

Population

There were 5120 infants ≥36 weeks GA admitted to UK neonatal units who were diagnosed with moderate/severe HIE and underwent management with TH. An additional 127 infants, diagnosed with moderate/severe HIE did not undergo TH and died within the first 48 hours of life, were included in the analysis. In total, 188 infants were excluded (Figure 1) leaving 5059 infants for analysis. Prior to matching, 2364 (46.7%) infants were born in a non-CC (Table 1).

In the unmatched groups, 65.4% of infants in the non-CC group were born in level 2 units, whereas 71.9% of the CC infants were born in a level 3 unit as expected with centralised care.

Figure 1. Flow diagram of study participants



GA, Gestational age; m/s HIE, moderate or severe hypoxic ischaemic encephalopathy; TH, therapeutic hypothermia; NHS, National Health Service

Table 1. Demographic and clinical variables of infants ≥ 36 weeks gestational age with moderate or severe hypoxic ischaemic encephalopathy both pre and post-propensity score-matching

	Unmatched infants				Propensity score-matched infants			
Variables	**Born in non-CC n=2364[a]**	**Born in CC n=2695[a]**	**SMD**	**p value[b]**	**Born in non-CC n=2165[a]**	**Born in CC n=2165[a]**	**SMD**	**p value[b]**
Gender (Male)	1354 (57.3)	1417 (52.6)	-0.095	0.002	1203 (55.6)	1225 (56.6)	0.020	0.49
Birthweight (grams)	3340 (2957-3760)	3332 (2940-3750)	0.014	0.70	3340 (2944-3760)	3340 (2970-3775)	-0.018	0.23
Gestation (weeks)	40 (38-41)	40 (38-41)	-0.015	0.47	40 (38-41)	40 (38-41)	-0.006	0.85
Birth year	2014 (2012-2015)	2014 (2012-2015)	-0.089	0.02	2014 (2012-2015)	2014 (2012-2015)	0.026	0.40
Pre-eclampsia	113 (4.8)	120 (4.5)	0.016	0.58	98 (4.5)	97 (4.5)	0.003	0.92
Maternal infection	555 (23.5)	559 (20.7)	0.066	0.02	480 (22.2)	480 (22.2)	0.000	0.99
Mode of delivery								
Em CS no labour	405 (17.1)	449 (16.7)	0.013	0.66	375 (17.3)	357 (16.5)	0.022	0.48
Em CS in labour	682 (28.9)	787 (29.2)	-0.008	0.78	639 (29.5)	630 (29.1)	0.008	0.80
El CS no labour	20 (0.9)	28 (1.0)	-0.020	0.48	21 (0.95)	18 (0.81)	0.014	0.64
El CS in labour	7 (0.3)	6 (0.2)	0.014	0.61	7 (0.33)	6 (0.27)	0.011	0.72
Instrumental	358 (15.1)	514 (19.1)	-0.104	<0.001	355 (16.4)	310 (14.3)	0.060	0.05
Missing	100 (4.2)	84 (3.1)	0.059	0.04	80 (3.7)	74 (3.4)	0.014	0.65
Presentation								
Cephalic	1833 (77.5)	2206 (81.9)	-0.017	<0.001	1753 (81.0)	1734 (80.1)	0.022	0.47
Breech	154 (6.5)	205 (7.6)	-0.043	0.13	142 (6.6)	162 (7.5)	-0.036	0.20
Transverse	11 (0.5)	12 (0.5)	0.003	0.91	11 (0.62)	11 (0.5)	0.003	0.92
Other	20 (0.9)	17 (0.6)	0.025	0.37	15 (0.7)	15 (0.68)	0.004	0.90
Missing	346 (14.6)	255 (9.5)	0.160	<0.001	244 (11.3)	244 (11.3)	0.000	1.00
Intrapartum events	276 (11.7)	349 (13.0)	-0.039	0.17	265 (12.2)	257 (11.9)	0.011	0.72

Table 1. Demographic and clinical variables of infants ≥ 36 weeks gestational age with moderate or severe hypoxic ischaemic encephalopathy both pre and post-propensity score-matching (continued)

	Unmatched infants				Propensity score-matched infants			
Variables	**Born in non-CC n=2364[a]**	**Born in CC n=2695[a]**	**SMD**	**p value[b]**	**Born in non-CC n=2165[a]**	**Born in CC n=2165[a]**	**SMD**	**p value[b]**
Significant resuscitation	1364 (57.7)	1703 (63.2)	-0.112	<0.001	1312 (60.6)	1278 (59.0)	0.033	0.28
Apgar at 5 min	4 (2-5)	4 (2-6)	-0.010	0.56	4 (2-5)	4 (2-6)	-0.003	0.72
Grade of HIE								
Grade 2	811 (34.3)	1068 (39.6)	0.110	<0.001	779 (36.0)	760 (35.1)	-0.018	0.55
Grade 3	1553 (65.7)	1627 (60.4)			1386 (64.0)	1405 (64.9)		
Place of birth level[d]								
Level 1	670 (28.3)	0	-		615 (28.4)	0	-	-
Level 2	1546 (65.4)	701 (26.0)	0.861	<0.001	1425 (65.8)	566 (26.2)	0.868	<0.001
Level 3	18 (0.8)	1938 (71.9)	-2.198	<0.001	16 (0.8)	1551 (71.6)	-2.183	<0.001
Home	96 (4.1)	56 (2.1)	0.094	<0.001	82 (3.8)	48 (2.2)	0.094	0.002
Midwife led unit	34 (1.4)	0	-		27 (1.2)	0	-	-
L2-L3 Transfer[d]	1386 (58.6)	452 (16.8)	0.957	<0.001	1283 (59.3)	358 (16.5)	0.981	<0.001

CC, Cooling Centre; SMD, Standardised mean difference; NVD, Normal vaginal delivery; Em CS, Emergency caesarean section; El CS, Elective caesarean section; HIE, Hypoxic Ischaemic encephalopathy; L2, level 2; L3, Level 3

[a] Data are n (%) or median (interquartile range).

[b] Categorical data analysed using chi squared test; non normally distributed continuous data analysed using Mann-Whitney U test; matched data by paired t-test for continuous data or McNemars test or extensions thereof for binary and categorical data

[c] Compared to baseline – NVD

[d] Variables not included in propensity score matching

Propensity matched groups

Propensity score-matching (Supplementary Figure 1) yielded a total of 4330 infants (n=2165/group, Table 1). Following matching, the standardised differences of all demographic and clinical variables were small compared to the unmatched groups. Infants born in a CC had significantly higher rates of survival without seizures prior to discharge compared to infants born in a non-CC (35.1% vs 31.8%; OR 1.15, 95% CI 1.02–1.31; p=0.02) and significantly lower odds of seizures overall (60.7% vs 64.6%; OR 0.84, 95% CI 0.75-0.95; p=0.007). Mortality prior to discharge was similar between the groups (Table 2).

In matched infants, 76.2% (n=2068) of infants with seizures received anticonvulsant medication, 27.1% of these infants required escalation of treatment with additional anticonvulsant agents but this was not different between groups (Table 2). Compared to CC infants, those born in a non-CC required similar amounts of cardiorespiratory support but did require a longer length of hospital stay (Table 2).

Table 2. Outcomes of infants who were born in either a non-cooling or cooling centre after propensity score matching

Outcome variable	Born in non-CC n = 2165[a]	Born in CC n = 2165[a]	Odds Ratio (95% CI)	*p* Value
Survival without seizures	693 (31.8)	764 (35.1)	1.15 (1.02-1.31)	0.02
Seizures	1399 (64.6)	1314 (60.7)	0.84 (0.75-0.95)	0.007
Death	312 (14.4)	343 (15.8)	1.11 (0.93-1.31)	0.20
Day of death (Hours)				
< 24	65 (3.0)	81 (3.7)	1.26 (0.90-1.80)	0.17
24 -72	87 (4.0)	116 (5.4)	1.35 (1.01-1.83)	0.04
> 72	160 (7.4)	145 (6.7)	0.92 (0.72-1.17)	0.49
Anticonvulsants[b,c]				
None	349 (25.0)	296 (22.5)		-
1 drug	668 (47.3)	663 (50.5)	1.17 (0.96-1.42)	0.10
≥2 drugs	382 (27.2)	355 (27.0)	1.09 (0.88-1.36)	0.40
Respiratory support[b]				
Ventilated	1454 (67.2)	1515 (70.0)	1.15 (0.94-1.42)	0.17
HFOV	388 (17.9)	340 (15.7)	0.97 (0.76-1.24)	0.81
CPAP	95 (4.4)	104 (4.8)	1.21 (0.85-1.72)	0.28
Hypotension	983 (45.4)	982 (45.4)	0.99 (0.88-1.12)	0.96
PPHN	260 (12.0)	236 (10.9)	0.90 (0.74-1.08)	0.25
Nitric Oxide	214 (9.9)	236 (10.1)	1.02 (0.84-1.25)	0.79
Length of stay (days)	11.3 (7.3-18.9)	10.0 (6.5-16.4)	0.14 (0.05-0.36)	<0.001

CC, Cooling Centre, 95% CI, 95% Confidence interval, HFOV, High Frequency Oscillatory Ventilation; CPAP, Continuous positive airway pressure; PPHN, Persistent pulmonary Hypertension; HIE, Hypoxic ischaemic encephalopathy

[a] Data are n (%) or median (interquartile range)

[b] Compared to baseline – No anti-convulsant/No respiratory support

[c] Data are n (%) of infants with seizures

Level 2 births only

To minimise variations in expertise and practice, we performed a further paired matched analysis of all infants born in a level 2 centres only. Propensity score-matching yielded 1392 infants (n=696/group, Table 3). Evaluation of the matching process demonstrated small standardised differences between infant groups for demographic and clinical background variables (Supplementary Table 2). Matched infants born in a level 2 CC had significantly improved survival without seizures (38.8% vs 32.3%; OR 1.33, 95% CI 1.06-1.65; p=0.01), reduced risk of seizures

(57.6% vs 63.9%; OR 0.77, 95% CI 0.61-0.95; p=0.02), and fewer anticonvulsants compared to infants born in level 2 non-CC. Mortality was not significantly different between the groups.

Table 3. Outcomes of infants who were born in either a level 2 non-cooling or cooling centre after propensity score-matching

Outcome variable	Born in non-CC n = 696[a]	Born in CC n = 696[a]	Odds Ratio (95%CI)	*p* Value
Survival without seizures	225 (32.3)	270 (38.8)	1.33 (1.06-1.65)	0.01
Seizures	445 (63.9)	401 (57.6)	0.77 (0.61-0.95)	0.02
Death	105 (15.1)	90 (12.9)	0.84 (0.62-1.13)	0.25
Day of death (Hours)				
<= 24	25 (3.6)	22 (3.2)	0.86 (0.48-1.54)	0.61
24 -72	28 (4.0)	27 (3.9)	0.96 (0.56-1.65)	0.89
> 72	52 (7.5)	41 (5.9)	0.77 (0.50-1.18)	0.25
Anticonvulsants[b,c]				
None	97 (21.8)	116 (28.9)	1.45 (1.17-1.79)	0.02
1 drug	222 (49.9)	206 (51.4)	0.90 (0.71-1.13)	0.67
≥2 drugs	126 (28.3)	79 (19.7)	0.57 (0.42-0.78)	<0.01
Respiratory support[b]				
Ventilated	493 (70.8)	454 (65.2)	0.92 (0.64-1.32)	0.66
HFOV	108 (15.5)	145 (20.8)	1.34 (0.88-2.04)	0.17
CPAP	29 (4.2)	30 (4.3)	1.03 (0.56-1.91)	0.91
Hypotension	326 (46.8)	303 (43.5)	0.88 (0.71-1.08)	0.22
PPHN	97 (13.9)	95 (13.8)	0.98 (0.72-1.32)	0.88
Nitric Oxide	66 (9.5)	67 (9.6)	1.01 (0.71-1.45)	0.93
Transferred L2-L3	615 (88.4)	449 (64.5)	0.24 (0.18-0.32)	<0.001
Length of stay (days)	10.6 (7.0-17.5)	10.5 (6.8-17.7)	0.55 (0.13-2.40)	0.41

CC, Cooling Centre; 95% CI, 95% Confidence Interval; HFOV, High Frequency Oscillatory Ventilation; CPAP, Continuous positive airway pressure; PPHN, Persistent pulmonary Hypertension;
[a] Data are n (%)
[b] Compared to baseline - No anticonvulsants/No respiratory support
[c] Data are n (%) of infants with seizures

Admission temperature to CC

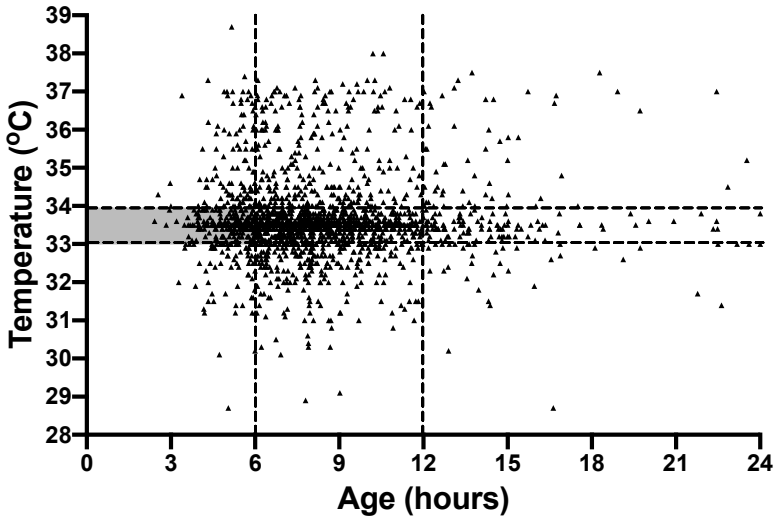
Out of the whole population, 2027 infants (85.7%) were transferred from a non-CC to a CC who had an admission temperature recorded shortly after arrival. Of these, 259 (12.7%) had a temperature on admission within the optimal therapeutic range of 33.5 +/- 0.5 ^{o}C (77) at 6 hours of age (Figure 2). There was no significant difference between time of admission for infants who arrived with their temperature within therapeutic range and those who were either over or under cooled (7 hours 40 minutes vs 7 hours 44 minutes, p=0.77).

Figure 2. Admission temperature and arrival time following transfer to a cooling centre (n=2027). (a) Tabular data (percentage) on each of the categories based on admission temperature and time, (b) Individual plots for each infant divided into key zones as in table in (a) with the shaded area optimal temperature in the 6 hour optimal therapeutic window

a)

		Age at Admission		
		<6 Hours	6-12 Hours	>12 Hours
Temperature	>34°C	4.2% (n=86)	11.9% (n=242)	2.4% (n=48)
	33-34°C	12.7% (n=259)	48.3% (n=979)	6.1% (n=124)
	<33°C	2.5% (n=52)	10.3% (n=209)	1.3% (n=27)

b)



Discussion

We evaluated the relationship between birth in a centre without active TH and the short-term outcomes of infants with HIE using propensity score-matching to balance important covariates. This nationwide UK study of over 5,000 infants with HIE, found almost half were born in non-CCs and were significantly less likely to survive without seizures compared to those born in CCs. This association appears to be driven by a higher rate of seizures in non-CC infants as the mortality rates were similar. The association remained when comparing only infants born in level 2 centres and following sensitivity analysis excluding those born at home or midwife led units. In

the UK, the management of moderate/severe HIE in infants ≥36 weeks GA with TH has been effectively and safely implemented resulting in a significant decrease in mortality and neurodisability (4-7). However, active TH remains mostly in centralised centres within geographical networks and therefore is not currently available in every birthing hospital, potentially delaying optimal treatment.

Neonatal seizures occur in approximately 50 – 60% of infants with HIE (38) and these infants are at an increased risk of adverse neurodevelopment outcomes, cerebral palsy and epilepsy compared to those with do not have seizures (39). There is increasing evidence that seizures, and the seizure burden relating to HIE, can have an additive effect on the initial brain injury and this could be independent of HIE severity (16-18, 24, 40). The Cochrane systematic review of TH for HIE found a borderline risk ratio (0.91; 0.83-1) in the reduction of seizures in infants undergoing TH compared to non-cooled infants (21). In agreement with this, and other smaller observational studies (23, 41), we found a significant reduction in seizures in those with access to immediate active TH in CCs, supporting the hypothesis that additional neuroprotection is provided by timely cooling. There was no increase in the use of anticonvulsants between the groups in the main analysis, although use was significantly increased in the subgroup of level 2 births potentially reflecting an increase seizure burden. These infants were also exposed to multiple anticonvulsants, some of which are thought to be neurotoxic and could have an adverse additive effect on long-term neurodevelopment (42).

Brain injury following hypoxic-ischaemic injury is progressive consisting of primary, latent and secondary phase during which excitotoxicity, release of free radicals, cell

inflammation and death occur (43). The latent period is considered the optimal time for TH through reduction of cellular injury (42, 43). Animal studies have shown that TH commenced in the late latent phase resulted only in partial recovery in cell survival compared to early use where cell injury and death was significantly reduced (11). Furthermore, TH commenced after the latent phase (~8.5 hours post injury) post seizure activity did not improve cell survival (46). The retrospective nature of this study means we cannot exclude other unmeasured potential confounders such as the quality of perinatal care in each centre. However, the association remained following subgroup analysis of level 2 centre births only, minimising some of the perinatal care practices. In the UK, level 2 centres are the most common maternity and neonatal hospitals, and they must have immediate access to newborn life support trained staff as well as the ability to provide initial newborn intensive care.

Essential to the delivery of centralised neonatal intensive care is the provision of rapidly mobilising, well-equipped transport teams to move infants in a timely and safe manner. During the period of this study, neonatal transport teams transitioned from mainly passive cooling to active, servo-controlled TH (47). Only 12.7% of infants transferred to a CC had an admission temperature within the optimal therapeutic range by 6 hours of age. A further 48.3% arrived between 6-12 hours of age in target range. Of note, only 3 of the 2027 transported infants had a recorded admission temperature in the range 33-34^{o}C within 3 hours of age. The database does not allow differentiation of equipment used by transport teams nor the time to achieve target temperature which could be before admission to the CC. Additionally, there could be delay in deciding to initiate TH in some of the infants which could account for those in the normothermic range. It is plausible that these infants only receive

partial protection from TH through passive cooling, which is more likely to occur during the late latent phase or beyond and result in decreased dampening of excitatory neurotransmitters (11). Noxious stimuli such as noise and vibration (48), experienced during transportation, result in cardiovascular instability (49, 50) and neuronal injury in animal models (51), which could contribute to this on-going brain injury pathway. Extremely preterm infants transferred in early life have an increased risk of severe brain injury (26, 27). It is unclear if exposure to significant levels of vibration and noise during inter-hospital transport, beyond that deemed safe for healthy adults, is a contributory mechanism in those infants or the additional seizures identified in this study.

Strengths

Our study is one of the largest nationwide population studies to date in the TH era utilising routinely collected individual patient data inputted on a daily basis from over 180 centres. This overcomes the single-centre study weakness by allowing variation in management of these infants across a whole healthcare service. The use of a robust propensity matching with well-balanced groups is also a significant strength, especially as undertaking a clinical trial of this nature would not be ethical or feasible. The size of the dataset has allowed identification of the reduction in seizure risk that may not have been possible with smaller studies.

Limitations

The main limitations of our study include the lack of neuroimaging results or long-term follow-up as these are not universally recorded in the NNRD dataset. However, previous work has demonstrated an association with seizures, MRI findings and later

development (41, 52). Another limitation is the lack of seizure details beyond the diagnostic coding, this would be useful to examine the burden of seizures. We did try to mitigate this with the use of anticonvulsant therapy. As highlighted, we cannot include local provision of maternal and neonatal care in the model as these data are not collected, although sub-analysis of level 2 births does address some of these issues. Criteria for TH in infants with HIE are clearly defined by national guidance (37) but the database had significant numbers of missing data such as 10 minute Apgar and cord/admission pH values. We therefore assumed if the diagnostic classification were made and an infant underwent TH they met the criteria for treatment.

Conclusions

Using well matched groups, this study demonstrates infants born with a diagnosis of moderate/severe HIE in a centre without active TH are less likely to survive without seizures prior to discharge compared to infants born in cooling centres. Furthermore, many infants transferred to cooling centres appear to arrive outside the target therapeutic time and temperature windows. The disparity of immediate access to active TH, known to reduce mortality and disability, in almost half of affected infants could delay optimal therapy and impact on outcomes. With the increasing recognition of the relative safety of TH (21), especially during the initial crucial 6 hour therapeutic window, consideration should be given for training and equipping non-CCs with active TH, thus avoiding delays in initiating and achieving optimal target temperature or the adverse effects of over or under-cooling. Further work is required to explore the potential adverse impact inter-hospital transport has on neonatal patients at risk of severe brain injury and what strategies could mitigate against this.

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Conflicts of interest

The authors have no conflict of interest. The sponsor had no involvement in the conduct of this study.

Data Access, Responsibility and Analysis

Dr Don Sharkey had full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

Data Sharing Statement

All data were extracted and supplied by the NDAU and are available from the corresponding author on reasonable request and with permission of the study team and NDAU.

Authors' contributions

LJS and DS made substantial contributions to the concept, planning, design of the study and acquisition of data. AM and DS collated access to TH data. LJS and DS analysed and interpreted the data with support from Professor C Coupland (acknowledgement). All authors assisted in drafting and editing the manuscript. All authors approved the final version for publication.

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Supplementary Online Content

Association of birth in centres without active therapeutic hypothermia and seizure-free survival following neonatal hypoxic ischaemic encephalopathy: A nationwide study

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Content:

eTable 1. Description of data fields used for determining covariates and outcomes from the National Neonatal Research Database

Principle Diagnosis at Discharge database entries for HIE Grading

Severe HIE :

- HIE Grade 3 - Severe Neonatal Encephalopathy
- Hypoxic ischaemic brain damage ; Severe
- Severe perinatal asphyxia (with 1 minute Apgar <4)
- Severe Neonatal Encephalopathy - Gr.3
- Hypoxic Ischaemic Encephalopathy (Gr 3)
- Severe Neonatal Encephalopathy – Grade 3 HIE

Moderate HIE:

- HIE Grade 2 - Moderate Neonatal Encephalopathy
- Hypoxic ischaemic brain damage ; Moderate
- Moderate perinatal asphyxia (with 1 minute Apgar 4-7)
- Moderate Neonatal Encephalopathy - Gr.2
- Hypoxic Ischaemic Encephalopathy (Gr 2)
- Moderate Neonatal Encephalopathy - Grade 2 HIE

Mild HIE:

- HIE Grade 1 - Mild Neonatal Encephalopathy
- Hypoxic ischaemic brain damage ; Mild
- Mild perinatal asphyxia (with 1 minute Apgar >7)
- Mild Neonatal Encephalopathy - Gr.1
- Hypoxic Ischaemic Encephalopathy (Gr 1)
- Mild Neonatal Encephalopathy - Grade 1 HIE
- Very mild perinatal asphyxia - clinically normal by 24 hours

Unspecified

- Birth Asphyxia
- Anoxic Brain Damage

Therapeutic Hypothermia

- Therapeutic Hypothermia
- Therapeutic Hypothermia (whole body cooling)
- Hypothermia Therapeutic

Principle procedures during stay entries for identification of Therapeutic Hypothermia

Therapeutic Hypothermia

- Therapeutic Hypothermia
- Therapeutic Hypothermia (whole body cooling)
- Hypothermia Therapeutic

Demographic and Clinical Variables

- Gender
- Birthweight
- Gestation in weeks
- Year of Birth
- Onset of Labour
- Presentation of fetus
- Mode of Delivery
- Methods of resuscitation
- Apgar score at 5 minutes

- Nulliparity determined from data field “Number of previous pregnancies” - Meconium stained liquor - Maternal Infection determined from data fields “Maternal pyrexia in labour” and “Problems during pregnancy with mother” (maternal UTI /chorioamnionitis/prolonged rupture of membranes) - Hypotension determined from data fields “Inotropes given”, “Daily drugs”, “Principal diagnosis at discharge” - Place of birth NHS hospital code - Place of birth NHS hospital level - Nitric oxide determined from data fields “Pulmonary vasodilator” and “Principle diagnosis at discharge” - Persistent pulmonary hypotension determined from data fields “Principle diagnosis at discharge” - Pre-eclampsia determined from data field problems during pregnancy with mother - Gestational Diabetes determined from data field “Problems during pregnancy with mother” - Acute intrapartum events determined from data fields “Problems (obstetric) during pregnancy with mother” (Reduced fetal movements/placental abruption/cord problems/shoulder dystocia) and “Principle diagnosis on discharge” - Significant resuscitation determined from data field “Methods of resuscitation” (cardiac compressions/intubation/adrenaline/other drugs) - Respiratory Support device (No support/CPAP/Ventilated/HFOV)
Outcome Variables
- Death determined from Destination at Discharge data field. If an infant was discharge “home”, “ward” or “Foster Care” the infants was coded as surviving. - Seizures determined from “Convulsions today” and “Principle diagnosis at discharge data fields”. Infants were also coded as having seizures if they received anticonvulsants in the “Daily Drugs” data field. - Number of anticonvulsants determined from “Daily Drugs” data field - Admission temperature - Time of admission temperature - Admission time to neonatal unit - Length of stay determined from Admission time and Time of discharge data fields

HIE, Hypoxic Ischaemic Encephalopathy; UTI, Urinary Tract Infection; NHS, National Health Service; CPAP, Continuous positive airway pressure; HFOV, High frequency oscillatory ventilation

eMethods

Propensity Score Matching

Propensity score analysis entailed fitting a logistic regression model using a stepwise process and evaluation of potential interactions to determine variables to include in the propensity score. For variables with missing data values were either imputed using Markov chain Monte Carlo method (1) or a separate category was defined indicating non-response. Initially, a priori background variables (gender, gestation, birthweight and birth year) were selected for inclusion into the logistic regression model regardless of their statistical association with the outcome. This baseline model was fitted with addition of one other background variable at a time. The variable with the greatest t ratio was included in the model if >1.0. This process was repeated, until t ratios for all remaining variables were smaller than 1.0. Following this analysis 13 covariates were included. These were:

- Gender
- Birthweight
- Gestation in weeks
- Birth year
- Nulliparity
- Pre-eclampsia
- Maternal infection
- Mode of delivery
- Presentation at birth
- Acute intrapartum events

- Significant resuscitation at birth
- Apgar score at 5 minutes
- Grade of HIE

Interactions

Interactions were determined for inclusion into model by ranking the variables in the concluding model by their t ratio and expanding this model by one second-order term at a time. The interaction term was included in the model if t ratio was greater than 2.71, which corresponds to nominal statistical significance at 10% level. No interactions reached this level in our analysis (2).

Propensity scores were estimated using logistic regression based on the selected background variables. A matched sample of untreated and treated infants was created through matching on the logit of the propensity score with caliper width set at 0.05. The propensity strata were trimmed by excluding infants with extreme propensities to minimise residual confounding. A nearest neighbour 1:1 matching algorithm with no replacement was used to form pairs of untreated and treated infants (3).

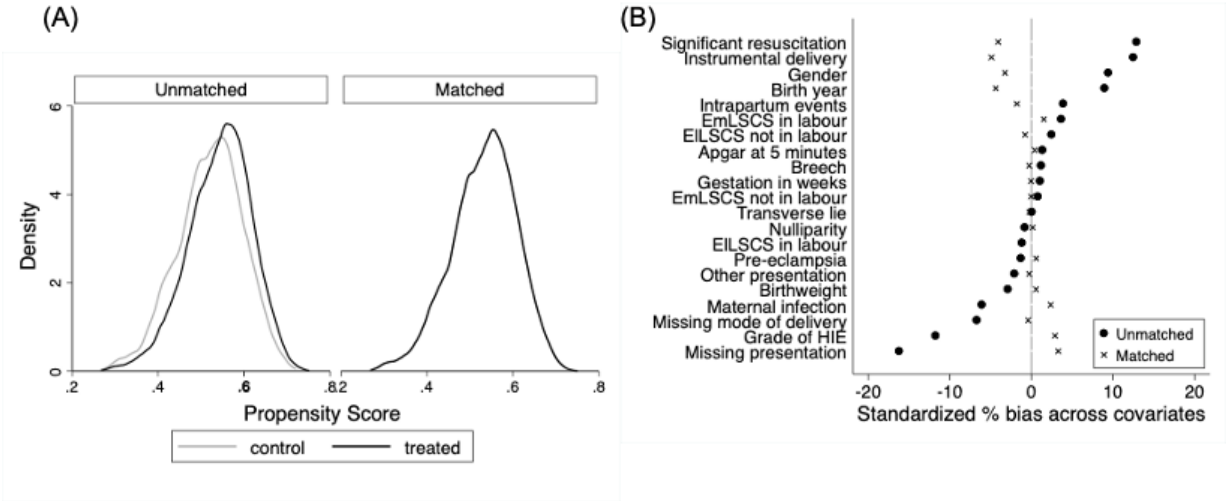
Assessment of balance

The matching process was evaluated by division of the propensity score into an optimal number of blocks and assessing the within-block equality of means of covariates across the treatment groups. The propensity distribution density was evaluated between control and treated groups both pre and post matching. (Supplementary Figure 2). Additionally, standardised percentage bias for each covariate (formulae as detailed by Rosenbaum and Rubin) after matching was calculated. A covariate was considered balanced if the standardised mean bias was <5% (4) (Supplementary Figure 2). The resulting model was well balanced with no covariate with a mean bias of >5% and overall mean bias 1.7 with no significant difference between the group ($p=0.75$).

Sensitivity analyses

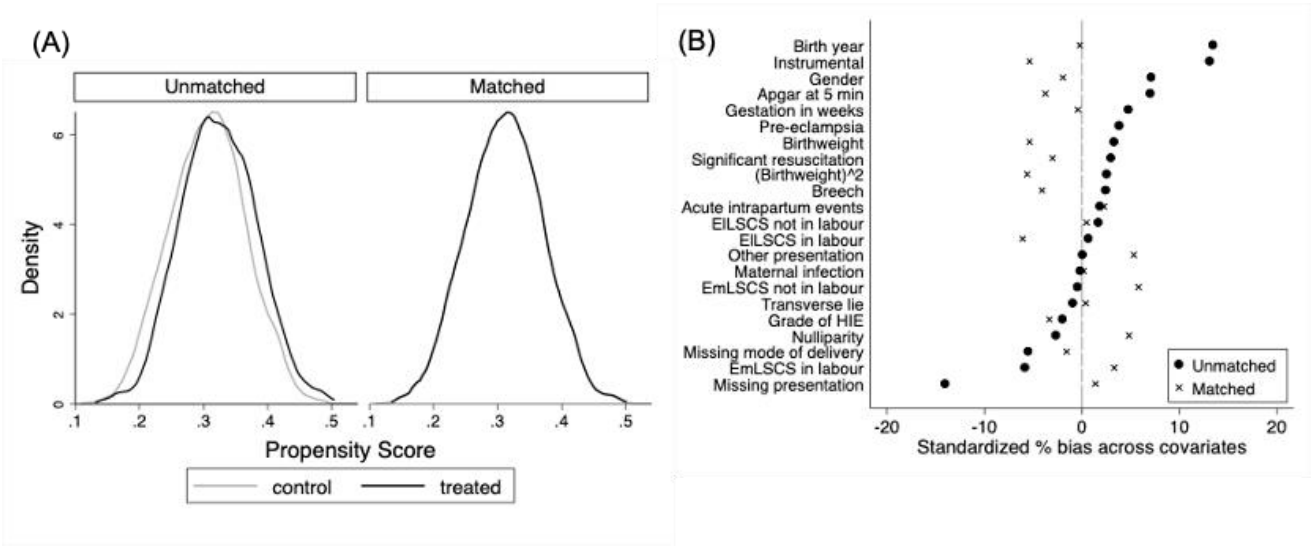
Sensitivity analyses were performed between infants born in level 2 non-cooling and cooling centres and between infants born in non-cooling and cooling centres but excluding infants born at home and midwife led units to mitigate potential bias. Further propensity score matching and assessment of balance was undertaken using the above described methodology for these subgroups of infants. Evaluation of model balance is demonstrated in Supplementary Figure 3 and 4.

eFigure 1. Propensity score distribution (A) and Standardised Bias (B) for infants born in non-cooling centres (control) and those born in cooling centres (treated) both before and after propensity score-matching



EmLSCS, Emergency caesarean section; ElLSCS, Elective caesarean section; APGAR, Appearance, Pulse, Grimace, Activity and Respiration score; HIE, Hypoxic ischaemic encephalopathy

eFigure 2. Propensity score distribution (A) and Standardised Bias (B) for infants born in level 2 non-cooling centres (control) and those born in cooling centres (treated) both before and after propensity score matching



EmLSCS, Emergency caesarean section; ElLSCS, Elective caesarean section; APGAR, Appearance, Pulse, Grimace, Activity and Respiration score; HIE, Hypoxic ischaemic encephalopathy

eTable 2. Demographic and clinical variables of infants born in level 2 centres pre and post propensity-score matching

	Unmatched infants				Propensity score-matched infants			
Variables	**Born in non-CC n=1546[a]**	**Born in CC n=701[a]**	**SMD**	**p value[b]**	**Born in non-CC n=696[a]**	**Born in CC n=696[a]**	**SMD**	**p value[b]**
Gender (Male)	877 (56.7)	373 (53.2)	-0.070	0.12	434 (61.9)	373 (53.6)	0.020	0.71
Birthweight (grams)	3332 (2929-3760)	3370 (2960-3730)	-0.033	0.42	3372 (3004 – 3764)	3370 (2953 – 3730)	0.036	0.50
Gestation (weeks)	40 (38-41)	40 (38–41)	-0.047	0.28	40 (39–41)	40 (38–41)	0.032	0.56
Birth year	2014 (2012-2015)	2014 (2012-2015)	-0.134	0.003	2014 (2013–2015)	2014 (2012–2015)	0.010	0.86
Pre-eclampsia	71 (4.6)	38 (5.4)	-0.040	0.40	31 (4.5)	38 (5.5)	-0.046	0.39
Maternal infection	383 (24.8)	173 (24.7)	0.002	0.96	176 (25.3)	172 (24.7)	0.013	0.80
Mode of delivery[c]								
Em CS no labour	263 (17.0)	118 (16.8)	0.005	0.92	115 (16.5)	118 (17.0)	-0.012	0.83
Em CS in labour	494 (32.0)	205 (29.2)	0.059	0.20	200 (28.7)	205 (29.5)	-0.016	0.77
El CS no labour	13 (0.8)	7 (1.0)	-0.017	0.71	7 (1.0)	6 (0.9)	0.015	0.78
El CS in labour	6 (0.4)	3 (0.4)	-0.006	0.89	4 (0.6)	3 (0.4)	0.020	0.71
Instrumental	240 (15.5)	144 (20.5)	-0.131	0.003	137 (19.7)	140 (20.1)	-0.011	0.84
Missing	57 (3.7)	19 (2.7)	0.055	0.24	28 (4.0)	19 (2.7)	0.072	0.18
Presentation								
Cephalic	1231 (79.6)	586 (83.6)	-0.103	0.03	588 (84.5)	582 (83.6)	0.024	0.66
Breech	84 (5.4)	42 (6.0)	-0.024	0.59	34 (4.9)	41 (5.9)	-0.044	0.41
Transverse	10 (0.7)	4 (0.6)	0.010	0.83	4 (0.6)	4 (0.6)	0.000	1.00
Other	11 (0.7)	5 (0.7)	-0.000	1.00	3 (0.4)	5 (0.7)	-0.038	0.48
Missing	210 (13.6)	64 (9.1)	0.141	0.003	67 (9.6)	64 (9.2)	0.015	0.78
Intrapartum events	187 (12.1)	434 (61.9)	-0.018	0.69	93 (13.4)	89 (12.8)	0.017	0.75

eTable 2. Demographic and clinical variables of infants born in level 2 centres pre and post-propensity score-matching (continued)

	Unmatched infants				Propensity score-matched infants			
Variables	**Born in non-CC n= 1546[a]**	**Born in CC n = 701[a]**	**SMD**	**p value[b]**	**Born in non-CC n = 696[a]**	**Born in CC n = 696[a]**	**SMD**	**p value[b]**
Significant resuscitation	935 (60.5)	434 (61.9)	-0.029	0.52	440 (63.2)	432 (62.1)	0.023	0.66
Apgar at 5 min	4 (2-5)	4 (2-6)	-0.070	0.09	4 (3 – 6)	4 (2 – 6)	0.018	0.74
Grade of HIE Grade 2 Grade 3	521 (33.7) 1025 (66.3)	243 (34.7) 458 (65.3)	0.020	0.66	236 (33.9) 460 (66.1)	240 (34.5) 456 (65.5)	0.012	0.82

CC, Cooling Centre; SMD, Standardised mean difference; NVD, Normal vaginal delivery; Em CS, Emergency caesarean section; El CS, Elective caesarean section; HIE, Hypoxic Ischaemic encephalopathy; L2, level 2; L3, Level 3

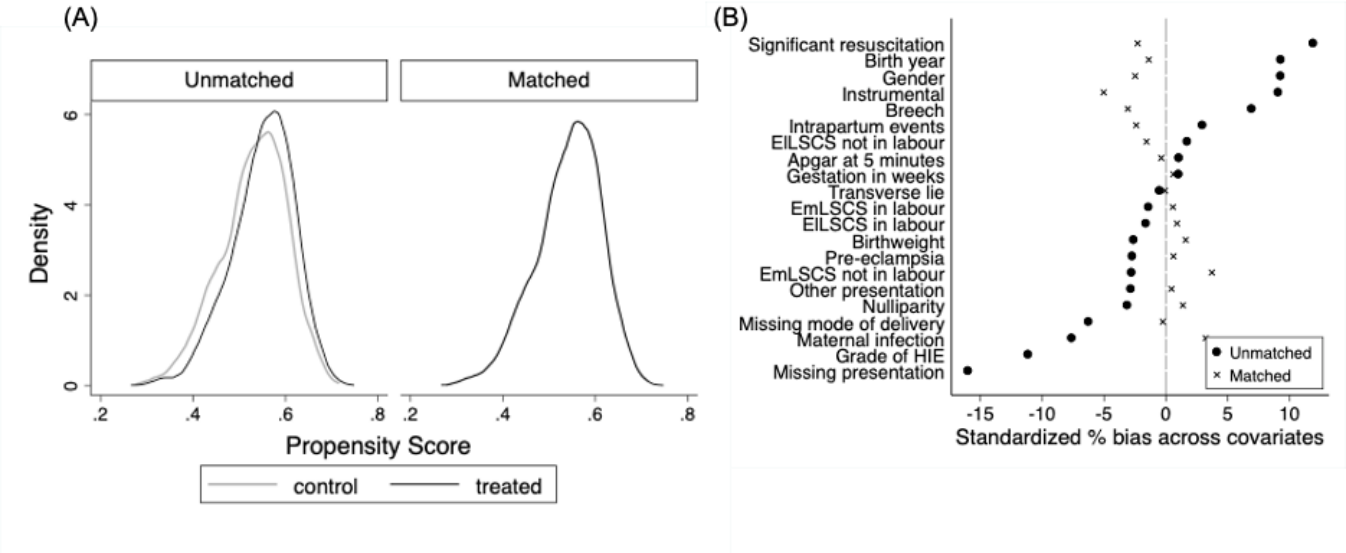
[a] Data are n (%) or median (interquartile range).

[b] Categorical data analysed using chi squared test; non normally distributed continuous data analysed using Mann-Whitney U test; matched data by paired t-test for continuous data or McNemars test or extensions thereof for binary and categorical data

[c] Compared to baseline – NVD

[d] Variables not included in propensity score matching

eFigure 3. Propensity score distribution (A) and Standardised Bias (B) between infants born in a non-cooling centre (control) and those born in a cooling centre (treated) both before and after propensity score-matching excluding those born at home or in midwife led units



EmLSCS, Emergency caesarean section; ElLSCS, Elective caesarean section; APGAR, Appearance, Pulse, Grimace, Activity and Respiration score; HIE, Hypoxic ischaemic encephalopathy

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