SERENITY: SEveRE Neurological Impairment
and children with medical complexity

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A thesis submitted to the school of Postgraduate Studies, Faculty of
Medicine and Health Sciences,
Trinity College Dublin, in fulfilment of the degree of Doctorate of
Philosophy

2022

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Declaration

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Summary

Background and aims
Children with Severe Neurological Impairment (SNI) have a significant disability as a result of a global impairment of the nervous system. They may be considered to be a subgroup of children with more general medical complexity but have unique challenges. There is no accepted definition of SNI and therefore a paucity of research into the medical and social needs of these children and their families. There is evidence to suggest that children with SNI may have an altered inflammatory state and immune function, but there is the potential for dysfunction in almost every organ system. Extra healthcare needs may place additional pressures on the families of children with SNI, for example, financial stress and mental health difficulties. We aim to create a consensus-based definition of SNI, quantify multi-system involvement in these children, with a particular focus on inflammation and immunity, and examine their wellbeing and that of their families.

Methods
The Delphi method was used to reach a consensus-based definition of SNI over 3 rounds of questionnaires with feedback to the multi-national and multi-disciplinary participants between each round. Cytokines were measured using ELISA at baseline and following stimulation with lipopolysaccharide (LPS). Flow cytometry was performed to assess the proportions and activation in response to LPS of lymphocytes, neutrophils and monocytes. Real-time PCR was used to measure expression of components of the NLRP-3 inflammasome. Blood samples were collected to assess biomarkers of cardiac and renal dysfunction, and multi-organ dysfunction was quantified using clinical outcome measures. The Pediatric Quality of Life and Caregiver Priorities & Child Health Index of Life with Disabilities questionnaires were used to assess wellbeing of the child with SNI and their families. A focus was placed on the effect on siblings of living with a brother or sister with SNI through the use of a focus group with teenage siblings.

Results
Thirty-four multi-disciplinary expert panellists from 5 countries participated in the Delphi process. Six items reached the 70% threshold for consensus and were included in a finalised definition of SNI. Children with SNI had alterations in several cytokine responses to LPS, with GM-CSF and IL-6 showing relative hyporesponsiveness and EPO being relatively hyperresponsive. Children with SNI had lower proportions of T cells, in particular those which were CD8+, and monocytes. Children with SNI exhibited CD66b hyporesponsiveness in neutrophils and TLR-4 hyper-responsiveness in monocytes. Children with SNI exhibited trained immunity with reduced expression of NLRP-3 and IL1β.
genes in response to stimulation with LPS. Creatinine was significantly lower in children with SNI than controls, reflecting their lower muscle mass. Other markers of renal function were not significantly different to controls. Troponin T and NT-proBNP were not significantly different in children with SNI. The children who participated had a median of 4 systems involved and were exposed to polypharmacy with a mean number of medications of almost 7. Families of children with SNI are significantly impacted by their child’s disability. Sibling questionnaires did not show differences in markers of wellbeing but a teenage focus group exhibited the complexities of living with a brother or sister with SNI.

Conclusion
We have created a consensus-based definition of SNI which we believe will improve consistency and quality of research for this group of children. Children with SNI exhibit alterations in cytokine and immune cell responses to stimulation with LPS. This raises the possibility of targeting various cytokines to improve neurological outcomes or reduce infection-related morbidity and mortality in this population. Children with SNI do not exhibit biochemical markers of renal or cardiac dysfunction but they have multi-organ dysfunction with considerable healthcare needs. We propose a multi-organ dysfunction scoring system to assist with clinical monitoring and objective measurement of outcomes in research. An understanding of the impact of caring for a child with SNI on families and their unmet needs may allow for more holistic care of these children. Siblings are also impacted by their brother’s or sister’s disability. Interventions to improve communication and facilitate peer support may help them to deal with the extra challenges they experience.
Acknowledgements

Firstly, I would like to sincerely thank my supervisors, Professors Eleanor Molloy and Denise McDonald, for their support, advice and patience over the past number of years, not just in my academic endeavours, but in all other aspects of my professional development.

I would also like to say a special thanks to Dr Johana Isaza-Correa, Dr Lynne Kelly and Dr Ashanty Melo for teaching me the ropes in the lab with patience and kindness. Their knowledge and support have been invaluable.

Thank you to Professor Maria Brenner and Dr Julie Hauer, for their assistance and expert advice in conducting the Delphi process. Thanks also to the expert panellists who participated in the process.

A special thanks to Dr Katie Hill for providing me with her expert knowledge, methodological advice, technical support, and assistance with the analysis of the focus groups for teenage siblings. I couldn’t have done it without your help. Thank you to Dr Patricia Byrne for providing her expertise and assistance in conducting the focus groups also.

Thanks to Ms Stephanie Kelly, CNS, the phlebotomy department and all of the staff in CHI at Tallaght for their logistical support in performing this work.

I appreciate the financial support provided by the National Children’s Research Centre, which was invaluable to me in conducting this research.

To my colleagues and friends in the Discipline of Paediatrics, Trinity College Dublin, thank you for the cups of coffee, conversations, laughs, advice and support. It has meant so much to me over the past number of years and has made the journey a lot more pleasant.

A massive thank you to my wife, Áine, for her unwavering support, patience, love and friendship. This would not have been possible without you! Thanks to my two boys, Fionn and Odhran, for all the fun and for reminding me of the joy of living in the moment. Special thanks to my parents for their love and encouragement throughout the years.

Finally, to the children, young people and families who participated. Your spirit in the face of significant adversity is truly awe-inspiring. Thank you
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Chapter 1 – Introduction
Children with complex, chronic health conditions represent a hugely important subgroup of children who interact with healthcare professionals. The conditions with which they present can be varied and a number of terms have been developed with which they can be classified. These classifications can be useful to assist with quantifying the impact of these disorders on the child, the family, the healthcare system and society. Children with Special Healthcare Needs (CSHCN); Children with Medical Complexity (CMC); Children with Chronic Conditions (CCC); Medically Fragile Children; Neurodisability; and Neuromuscular Disorders are some of the terms used. Each describes a slightly different population, some larger than others, and with some overlap between the groups. The value of these descriptors lies in their ability to cohort children with similar traits together, which is useful when communicating, both in the research and clinical setting. Severe Neurological Impairment (SNI) is another such term which is commonly used but which has not been previously defined.

CSHCN is probably the most broad classification of children with chronic medical conditions, being defined as “…those who have or are at increased risk for a chronic physical, developmental, behavioral, or emotional condition and who also require health and related services of a type or amount beyond that required by children generally”(1). Amongst these children, those with neurological conditions have more unmet needs overall, and those with multiple neurological conditions report “greater dissatisfaction with care, care coordination, ability to get a referral and ability to use other services”(2).

Within the category of children with neurological conditions, there is a wide and heterogeneous array of disorders. We have chosen to focus on children with SNI, who have significant needs. Their spectrum of needs are unique to this population and occur as a direct result of their neurological impairment. There may be similarities between those with SNI and neuromuscular disorders, such as Duchenne Muscular Dystrophy, but there are also important differences including: differences in risks and benefits of certain interventions; autonomic dysfunction; central neuropathic pain; increased risk of epilepsy; and precocious puberty(3). With this research, we have, therefore, endeavoured to contribute to the knowledge regarding this important group of children.

1.1 The definition of Severe Neurological Impairment

Severe Neurological Impairment (SNI) is a term that is commonly used in the medical literature. However, there is no internationally agreed definition of SNI. Several issues remain to be clarified such as the disorders of the neurological system which are included; whether disorders of the central nervous system (CNS), peripheral nervous system (PNS) or both are included; whether SNI should be described in terms of functional limitation; and the domains of functional impairment that are most important to the classification.
SNI is not a diagnosis, but a descriptive term for a group of patients with similar healthcare conditions, care needs and challenges. However, there are no agreed criteria for inclusion in this group, and therefore no opportunity to capture prevalence. This limits the national and international opportunities for research into healthcare needs, treatment opportunities, resource planning and outcome.

While children with SNI have much in common with other medically complex children, they are a unique and vulnerable cohort of patients with their own needs and challenges. Significant neurological impairment is the consistent feature, resulting in impairment of intellectual function, mobility and communication, and multiple specific medical issues.

SNI covers a range of diagnoses, some relatively common, some extremely rare and some disorders where no definitive diagnosis has been made. The value in considering children with SNI under a common umbrella lies in the fact that it can help in decision-making and planning for the future, particularly in cases where there is a lack of information on a particular disorder or where there is no diagnosis. In these cases, phenotypic descriptions, such as SNI, can be very useful.

1.1.1 The importance of definitions and consistency in research

Several other authors also argue that there is importance in adequately defining the terms that we use to describe various groups (4, 5). A quote attributed to Plato effectively summarises this argument: “The beginning of wisdom is the definition of terms”. Evidence to objectively show that the lack of a definition of SNI is actively harming or causing distress to patients and their families does not exist. However, one could argue that the lack of an internationally-agreed definition limits the reliability of research in this area. The importance of consistency in healthcare research is further demonstrated by the existence of the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) network. This is an international initiative which seeks to improve the value of healthcare research by ensuring transparent and accurate reporting (6). We argue that using an agreed definition is one of the most basic ways of ensuring that this goal is achieved.

1.1.2 The Delphi Process

The Delphi process was originally developed in the 1950’s in the United States of America during the Cold War. It was adopted by the RAND Corporation, a research institution to gather the opinions of experts on the effects of technology and future developments in warfare. The technique was initially shrouded in secrecy and used primarily for military purposes. As a result, a description of the original experiment was only published 10 years following its development(7, 8).
The name of the technique originates from the Oracle at Delphi. The Temple of Apollo in Delphi was the most important shrine in Greece and was presided over by a high priestess who served as Oracle. Oracles were thought to provide wise counsel and prophecies of the future. The name may, therefore, seem appropriate, but at least one of the inventors of the technique believed that the name was unfortunate. Dalkey felt the term implied “something smacking a little of the occult – whereas, as a matter of fact, precisely the opposite is involved”(9).

The technique has evolved over the years and has been adapted in numerous ways e.g. the “Decision Delphi”(10). The core principles, however, have remained the same: anonymity of participants, the use of several rounds and the provision of feedback between rounds (11). The process has been used increasingly in the past 30 years and is now a widely accepted scientific method(7). It operates on the assumption that group decisions are more accurate than individual ones.

The Delphi process is useful in a variety of situations and is particularly useful when the problem at hand does not lend itself well to precise analytical techniques and where several issues must be explored and assessed(12). It therefore, is useful for reaching consensus on definitions of terms. It has been used repeatedly in the creation of definitions in healthcare(5, 13-17).

1.2 Multi-organ dysfunction in Severe Neurological Impairment

Children with SNI are a heterogenous group of patients with a wide variety of diagnoses, many of them rare. Several children with SNI may not have received a definitive underlying diagnosis. Despite this heterogeneity, all children with SNI are unified by their increased healthcare needs. However, to date, the extent of multi-organ dysfunction in children has not been quantified. There is no data to accurately quantify the prevalence or incidence of SNI. The lack of figures has implications for resource planning and research in the area.

Children with cerebral palsy (CP) are likely to make up a significant proportion of children with SNI. In common with SNI, CP is a clinical descriptive term which covers diverse conditions in terms of aetiology. However, CP is also heterogenous in the severity of the functional impairments and comorbidities that are linked with it. Thus, not all children with CP will have SNI. Nevertheless, the multiorgan dysfunction which is associated with CP, particularly for those with more severe motor impairment, is a reasonable starting point from which to consider the possible comorbidities that clinicians should be aware of when caring for children with SNI.
1.2.1 Multi-organ Dysfunction in Cerebral Palsy

Cerebral palsy (CP) describes a heterogeneous group of non-progressive disorders of posture or movement, causing activity limitation, due to a lesion in the developing brain (18). The worldwide prevalence of CP is approximately 2 per 1000 live births (19), although the most recent data from the Surveillance of Cerebral Palsy in Europe Network reports the prevalence as 1.64 per 1000 live births (20).

There are many recognised risk factors for cerebral palsy, including preterm birth, multiple gestation, congenital malformation, genetic and metabolic abnormalities, intra-uterine exposure to infection or inflammation, birth asphyxia, perinatal stroke and thrombophilia (21). CP has an overall rate of 13% in survivors of term neonatal encephalopathy (NE), and 24% of term children with CP have a history of moderate to severe NE (22). Neonatal asphyxia induces global hypoxic-ischaemia resulting in multi-organ injury (23). Early multi-organ dysfunction (MOD) in neonates with encephalopathy may persist in later childhood. Cardiac, renal, hepatic, haematological and neurological dysfunction are well described in neonates with NE, but follow-up in childhood is not routine practice.

Life-expectancy is reduced in children with CP, based on retrospective data collected between 1983 and 2010 in California (24, 25). Respiratory and cardiac dysfunction are well documented causes of increased morbidity and mortality in children and adults with CP and dysfunction in other organs such as the renal, gastrointestinal and haematological systems are gaining recognition.

Multi-organ dysfunction may be present to a certain degree in all children with CP but, within each organ system, it is known that with increasing motor impairment, as measured by the GMFCS, there is a higher risk of dysfunction. For example, lower levels of physical activity are associated with greater levels of endothelial dysfunction (26-28); increasing motor impairment is associated with reduced heart rate variability (29, 30); those with greater motor impairment are more likely to have lower urinary tract symptoms and urodynamic abnormalities (31); dysphagia occurs in up to 99% of individuals with “severe generalised CP and intellectual disability” (32); and, those with more severe CP are more likely to have obstructive sleep apnoea (33). Thus, SNI is likely to be associated with a significantly higher risk of multi-organ dysfunction. In those with syndromic causes of SNI, one should be mindful that there may be specific comorbidities associated with that particular syndrome and continue to monitor for these issues throughout the child’s lifespan.

Assessment of multi-organ function may be useful to ensure complete resolution of any abnormalities and avoid complications in later childhood or adulthood. It may also contribute to an improved understanding of the aetiology and thus may have an
implication in prevention, interventional methods and therapies. MOD in CP is summarised in Table 1.1.

1.2.2 Neurological & Developmental

Cerebral palsy, by definition, is attributable to a lesion in the developing brain(18). Periventricular white matter lesions are observed on magnetic resonance images (MRI) in 50% of children with CP, and cortical or subcortical grey matter lesions in 20%(34). However, in a study of children with CP born after 36 weeks’ gestation, neuroimaging studies were normal in one third of children(35). In the same study, the most common abnormality on neuroimaging was focal infarction, observed in 22% of the children. Other abnormalities noted were brain malformation, including schizencephaly, hydrocephalus, polymicrogyria, lissencephaly, agenesis of the corpus callosum, septo-optic dysplasia, and cerebellar anomalies. Periventricular leukomalacia (PVL) which is associated with prematurity was seen in 12% of the children, hypoxic ischaemic brain injury in 5% and intracranial haemorrhage in a further 5%(35). Grey and white matter lesion burden has been shown to correlate with motor and cognitive function(34). The American Academy of Neurology recommend neuroimaging in all children with CP. In those where history and imaging do not determine aetiology or where findings are atypical, genetic or metabolic investigations should be considered. Where a brain malformation is found, it is recommended that genetic and metabolic conditions also be considered(36).

Cerebral Palsy is the most common cause of motor disability in children(21). Studies of the epidemiology of CP traditionally group children with CP into phenotypic categories based on the type of tone abnormality and the distribution of limb weakness(35). The severity of motor impairment is assessed by the Gross Motor Function Classification System (GMFCS) and this is the most widely used standard measure of motor function(37). Aside from motor impairment, neuropsychological & cognitive function is commonly impaired in children with CP. Intellectual disability is seen in approximately one third of patients with CP, GMFCS I-II and two thirds of patients with CP, GMFCS III-V (38) and has previously been shown to be the strongest predictor of mortality in CP(22). Children with CP who have a history of neonatal encephalopathy (NE) are significantly more likely to develop cognitive impairment and have a greater burden of disability, including epilepsy, than children with CP who did not have NE(22).

The overall prevalence of epilepsy in children with cerebral palsy was found to be 38%, although this varies significantly depending on CP subtypes, aetiology and cognitive function(39). Prevalence increases with increasing severity of motor impairment. There is a higher prevalence of epilepsy in children with CP secondary to CNS malformations, CNS infections, and grey matter lesions than those with CP secondary to white matter changes or CP of unknown aetiology. Furthermore, the prevalence of epilepsy in CP
increased with decreased cognitive function(39). Age of onset of epilepsy can vary with type of CP, and early recognition and diagnosis is essential for prompt management. Management of seizure disorders are not significantly different in children with CP compared to those without, but care should be taken not to confuse seizures with dyskinesia (38). Behavioural issues are also more common in children with CP overall and especially with coexisting epilepsy(40). Hyperactivity and inattention are significantly higher in children with CP and epilepsy than in children with CP alone (40).

Hypertonia is a significant issue for many children with CP, leading to impaired motor function, pain, and difficulties with daily care. Hypertonia may manifest primarily as spasticity, dystonia or less commonly rigidity, alone or in combination(41). Spasticity management involves a multi-disciplinary approach, including individual, goal-oriented physiotherapy and occupational therapy(42). Options for medical treatment include diazepam, baclofen (oral or intrathecal) and botulinum toxin type A(42). In certain cases, Selective Dorsal Rhizotomy may be considered(42). Dystonia management requires specific medications, including trihexyphenidyl and gabapentin, but current evidence is limited, and the majority of care pathways rely on expert opinion(43, 44). Choice of treatment should be tailored to the individual and based on their treatment goals.

1.2.3 Hearing and Vision

The incidence of hearing impairment in children with CP is reported to be between 7 to 37.5%(45) with the following distribution: 48% conductive, 4% sensorineural, 25% mixed and 23% unspecified(45). Conductive hearing loss has been associated with a high rate of chronic otitis media, eustachian tube dysfunction, abnormal anatomy, and craniofacial anomalies in this cohort. Sensorineural hearing loss (SNHL) has been postulated to be associated with low birth weight, hyperbilirubinemia, and neonatal hypoxemia in this population, with more severe hearing loss in those with quadriplegia, epilepsy, or intellectual disability (45). Furthermore, congenital CMV infection is associated with cerebral palsy and is a leading cause of SNHL in children(46). Although there are inherent difficulties in testing this patient group for hearing loss using pure tone audiometry, it should not preclude thorough audiological assessment.

Children with cerebral palsy can be diagnosed with visual impairments that are ocular or cerebral in origin or combination of both(47). Cortical visual impairment (CVI), refractive errors and accommodative dysfunction are common in children with cerebral palsy(48). Periventricular Leucomalacia (PVL) on MRI is found to have a strong association with CVI(49). Dutton et al provide a questionnaire inventory as an effective means to assist the clinician in recognition of CVI so that the appropriate strategies and management can be put in place to assist the child (48). Lesions involving the basal ganglia are more frequently associated with impaired visual function than those involving
the visual occipital cortex(50). Furthermore, there is a correlation between the severity of basal ganglia lesions and the degree of visual impairment, with moderate and severe lesions of the basal ganglia always associated with visual impairment in one study(51). White matter lesions are not as reliable an indicator of neonates who will develop visual impairment, as only those with severe white matter changes had visual impairment and the presence or severity of visual impairment was not always associated with lesions involving the visual cortex. There was over 90% concordance between visual assessments performed in the first year of life and at school-going age (51), supporting the need for early and periodic screening and long-term monitoring.

1.2.4 Respiratory Dysfunction

Disorders of the respiratory system are a significant cause of morbidity(52) and are the largest cause of premature death in children and young people with CP(53-55). Respiratory symptoms are common, particularly in those with a GMFCS level of V and are most prevalent at meal-times(56). Respiratory diagnoses account for a high proportion of hospital admissions in this population(57, 58) and many of these admissions may be predictable and modifiable(58). Blackmore et al demonstrated that 12.1% of children and young people with CP were admitted to hospital due to respiratory illness over the course of 1 year(59). In the same period, 19.2% had been prescribed 2 or more courses of antibiotics for respiratory issues(59).

Reasons for the high rate of respiratory morbidity are complex, multifactorial and several factors, such as seizures, oromotor dysfunction, gastro-oesophageal reflux and scoliosis, may coexist within the same individual(59, 60). Impaired airway clearance, due to respiratory muscle weakness, impaired coordination and poor positioning, in the context of poorer motor function, may predispose to infection and atelectasis. Bronchopulmonary dysplasia may be an issue in children with CP who were born prematurely(52, 61).

Swallowing is inextricably linked with respiratory dysfunction. It is a complex process involving the brainstem, multiple overlapping cortical areas, sensory input from the oropharynx and a high level of coordination between the central and enteric nervous systems(62). Chewing and swallowing problems are significantly associated with poorer gross motor function and intellectual ability(63) and impact on respiratory health. Dysphagia is common, occurring in 43% of all children with CP(62) and in children with severe generalised CP and intellectual disability can be as high as 99%(32). This can result in video fluoroscopically-proven aspiration in up to 70% of children with severe CP(64). Aspiration is a significant cause of respiratory symptoms and pneumonia in CP and can result from both direct aspiration from the oropharynx or indirect aspiration of gastric contents(65). Aspiration may result in pneumonia through introduction of
pathogenic bacteria, alteration of the respiratory microbiome, promotion of airway inflammation or a complex interaction of all of these factors (66-70).

Lower respiratory tract infections (LRTI) in general are more common in children with CP and they have more severe disease requiring admission to the intensive care unit (71). Thorburn et al reported that 89% of children with moderate to severe CP (n=53, 90% with GMFCS ≥3) ventilated in the paediatric intensive care unit for 4 or more days carried abnormal bacteria flora, with *Pseudomonas* and *Klebsiella* being the most frequent. In children without CP (n=257) the carriage rates of abnormal bacteria were 55% with 10% resistant bacteria. Almost half of these were resistant bacteria and the mortality rate was significantly higher amongst the group with CP compared those without CP (17% versus 10%) (72). Bronchiectasis, which predisposes to recurrent LRTI, is reported in 67% of patients with CP presenting to a dysphagia clinic (73). Similarly, a retrospective study of 100 children with chronic pulmonary aspiration found that 66% had bronchiectasis, with the significant risk factors found to be severe neurological impairment (NI) (OR 9.45, 95% CI 2.05–43.6) and parent report of previous gastroesophageal reflux (OR 3.36 95% CI 1.08–10.43). In the cohort of children with severe NI (n=30), 93% had bronchiectasis (74).

Management of respiratory morbidity often requires a multifaceted approach. Those who care for children with CP must rule out aspiration when they present with respiratory symptoms. In particular, one must be cognisant of the fact that silent aspiration is more common in children with NI (75). In the respiratory context, some authors argue that gastroesophageal reflux (GOR) should be treated (73), presumably in an effort to reduce indirect aspiration of gastric contents. However, the evidence here is not conclusive. A randomised control trial of 38 children with asthma and GOR found no difference in respiratory symptoms or lung function between those treated with a proton-pump inhibitor (PPI) and placebo (76). Conversely, a prospective study comparing PPI versus histamine H2-receptor antagonists (H2RA) identified 35 out of 74 children with oesophageal and extra-oesophageal (respiratory and laryngeal) manifestations of GOR (77). Eighty-three percent had complete resolution of symptoms when treated with a PPI, compared with 35% in the H2RA group. Conflicting evidence is also seen with regards to the surgical treatment of GOR for respiratory symptoms. A prospective cohort study, comparing outcomes of Nissen fundoplication in children with NI (n=46) versus without NI (n= 42) showed a reduction in the proportion of children with NI who had more than 4 respiratory infections per year from 33% pre-operatively to 8%, 18% and 10% at 1, 2 and 3 year postoperative respectively (78). However, respiratory symptoms were not reported to be improved post-operatively. Another retrospective review of 34 patients following fundoplication, 15 of whom had NI, evaluated outcomes at 7 years post-operative. Pulmonary symptoms were frequent in children with NI pre-operatively (13/15) and did not significantly improve after surgery, while half of the children with NI developed
new symptoms or complications during the period of follow-up (79). A retrospective analysis of discharge data for 342 paediatric patients, found no difference in hospital admissions for aspiration pneumonia, other pneumonia or respiratory distress before or after anti-reflux surgery (80). Finally, a meta-analysis by Lauriti et al showed that gastrojejunal feeding was no better at preventing respiratory complications than anti-reflux surgery in children with NI (81). Large prospective, high quality trials would be useful to determine if and when treatment of GOR is useful in improving respiratory morbidity.

The British Thoracic Society (BTS) recommends the use of mucolytics, such as nebulised saline, to assist clearance of “tenacious secretions” in children with neuromuscular weakness (82). Physical therapies to help removal of respiratory secretions are likely to be helpful but may be a significant extra burden on families (73). Finally, there is no evidence for the use of prophylactic antibiotics in children with CP. Long-term azithromycin is recognised as beneficial in cystic fibrosis (83) likely secondary to its anti-inflammatory properties. The BTS recommend consideration of prophylactic antibiotics in non-cystic fibrosis bronchiectasis although the authors acknowledge that controlled trials are lacking (84). Prophylactic antibiotics are frequently prescribed for children with CP. Macrolide antibiotics have antibacterial, anti-inflammatory, pharmacokinetic and bioavailability characteristics that make them useful for prophylaxis, as well as being safe with few side effects (52, 73, 85). High quality randomised controlled trials are required to assess their effectiveness and indications for use in children with CP.

1.2.5 Sleep Problems

The prevalence of sleep disorders among children with CP is up to 36%, although this varies within subgroups, according to CP phenotype (bilateral spastic, 41%; unilateral spastic, 24%; dyskinesia, 30.8%; ataxia/non-classified, 17.4%) (86, 87) and can have a significant impact on quality of life (88). Total sleep disorders are principally associated with seizures (89). Subtypes of sleep disorders in children with CP include disorders of initiating and maintaining sleep (DIMS), sleep breathing disorders, sleep hyperhidrosis, sleep-wake transition disorders, disorders of arousal, parasomnias, and non-restorative sleep (86). DIMS are the most frequently occurring subtype of sleep disorder with symptoms including difficulty falling asleep and frequent night awakening (89, 90). This may be secondary to pain, involuntary movements, associated behavioural and adaptive difficulties, underlying brainstem dysfunction or severe visual impairment (89, 91-95).

Behavioural considerations are a significant cause of sleep disturbance in CP. In a study of children with moderate to severe motor disability (n=505, 216 had CP) which used parental questionnaires to evaluate sleep problems, 25% of children with CP had difficulties relaxing at night (96). A systematic review and meta-analysis by Horwood et al (86) calculated the rate of problems with “sleeping routines” as 51.3%, using the data
from a retrospective observational study of 154 children with CP. Interestingly, this concern decreased with worsening motor function from 100% in children classified as GMFCS I to 34% in children classified as GMFCS V and with increasing age.

The Sleep Disturbance Scale for Children (SDSC)(97, 98), Pediatric Sleep Questionnaire (PSQ)(99) and Childhood Sleep Habit Questionnaire (CSHQ)(100, 101) are validated tools used to document sleep problems in children and are frequently used in children with CP. Sleep affects the quality of life of these children(88, 102) and their families(103, 104) thus, sleep disorders are ideally evaluated in children with CP using these questionnaires and subsequently monitored and managed appropriately. Sleep hygiene is first-line therapy for sleep disturbance in all children including those with neurodevelopmental disabilities(105-107). This includes measures such as a consistent bedtime routine, a dark and quiet bedroom, limiting technology use before bed and independence in falling asleep (107, 108). Other therapy options for managing sleep disorders in children with neurodisability include behavioural strategies such as play-based therapy(109) and medications such as melatonin.

Recent research has provided interesting insights into some of the possible pathophysiological mechanisms that underlie sleep disturbance in CP. Circadian Locomotor Output Cycles Kaput (CLOCK) genes are rhythmically expressed in the pineal gland which secretes melatonin and is crucial for regulation of the sleep-wake cycle(110). Expression of CLOCK is altered following hypoxic-ischaemic brain injury in animal models(111) and alterations in circadian rhythm have been reported in children following even mild perinatal hypoxic-ischaemia(112). Day and night variability of melatonin has been found to be lower in children with CP(113). Melatonin is widely prescribed for sleep disturbances and has shown improvements in sleep latency and night-waking in these children(104, 114, 115). The National Institute for Health and Care Excellence (NICE) have produced a guideline on assessment and management of cerebral palsy in under 25-year-olds, within which guidance on managing sleep disturbances is provided(38). Sleep hygiene is recommended in all cases and melatonin where an identifiable cause of sleep disturbance is not found(38).

Sleep-disordered breathing is more common in children with CP than in their typically-developing peers(89, 116, 117), occurring in up to 18.1% of children with CP compared with 7.4% of controls(92). Children with more severe CP as measured by the GMFCS, as well as children with comorbid epilepsy have a higher risk of obstructive sleep apnoea (OSA)(33). OSA may be contributed to by hypotonia, glossoptosis, laryngomalacia, midface and mandibular abnormalities, adenotonsillar hypertrophy, gastro-oesophageal reflux, medications and brainstem dysfunction(117, 118). Children with CP may be at increased risk of central sleep apnoea (CSA) as neurological disorders are the commonest risk factor for this(119). Sleep-disordered breathing in CP may also be
caused by muscular weakness. Children with CP have reduced diaphragmatic mobility, respiratory muscle strength and chest expansion (120). Sleep disorders in general have a significant impact on quality of life in CP (88, 121) and therefore, treatment should be strongly considered for sleep disordered breathing. Non-invasive ventilation is a potential treatment. However, this can prove more challenging in children with CP and in a retrospective review, 55% of patients with CP failed to establish on this treatment compared with an overall failure rate of 8.7% in the same tertiary service (118). The American Academy of Pediatrics has recognised adenotonsillectomy as first line treatment for sleep apnoea in children (122). A randomised trial (n=464) showed benefits in reducing symptoms of OSA as well as improvements in behavioural, quality of life and polysomnographic measures (123). Surgery may be rendered more difficult in children with neurological impairment due to co-morbidities. A retrospective review of 375 children with OSA and syndromic or neurological comorbidities, 105 of whom had cerebral palsy, found that the average apnoea-hypopnoea index reduced from 12.4 to 5.7 ($p=0.002$) following tonsillectomy, leading the authors to conclude that adenotonsillectomy remained the mainstay of treatment for OSA in these children.

Sleep disorders can be difficult to diagnose in this population but can have a significant effect on the lives of children with CP and their families. It is important to be mindful of sleep issues and consideration should be given to regular screening for these disorders in children with CP.

1.2.6 Cardiac dysfunction

A cohort study which included 958 adults with CP reported that they had a three-fold increase in disorders of the circulatory system (124). In a linkage study involving the Victorian Cerebral Palsy Register and the Australian National Death Index, out of 3507 individuals with CP, 418 were known to have died between the years 1970 and 2010, with cardiac causes of death second only to respiratory (55).

Chronic inflammation is known to contribute to the formation of atherosclerosis, a common cardiovascular disorder (125) and children with CP are known to have altered inflammatory responses (126, 127) increasing the possibility that these children with CP may develop arterial pathology that leads to atherosclerosis in adult life. Children with CP are less physically active than their typically developing peers (128) and lower levels of physical activity are more likely to be associated with endothelial dysfunction (26-28). Early-stage atherosclerosis can be demonstrated by measuring the carotid intima media thickness (CIMT) of the carotid arteries using advanced ultrasound (129). Increased CIMT is predictive of atherosclerosis and future cardiovascular events (130). Carotid ultrasound assessment of children with CP (n=100), demonstrated increased CIMT when compared to controls (n=35), suggesting that children with CP are at increased risk of
atherosclerosis and coronary artery disease(129). Follow up studies of these children in later childhood and adulthood are required for prompt diagnosis and management. Heart rate variability (HRV) reflects autonomic control of the sinus node. Significant reductions in HRV have been found between children with CP and controls. Within children with CP, HRV significantly decreases with poorer motor function as measured by GMFCS(29, 30, 131). This indicates a less efficient and less adaptive cardiac autonomic system in children with CP(131) and reiterates the need for cardiac surveillance in those with CP, particularly those most severely affected.

Measurement of foetal HRV has also been raised as a possible tool for predicting the development of cerebral palsy. A retrospective review of 95 children with CP, born at term, compared data from foetal monitoring and compared them with controls. Late decelerations and reduced beat-to-beat variability were associated with a sharp increase in risk of developing CP(132). Reduction in HRV can improve the prediction of neurodevelopmental outcome in preterm infants (n=79) when combined with poor repertoire abnormal general movements (PR GMs) using Prechtl’s assessment method(133, 134).

Evidence for myocardial dysfunction in children with CP is lacking. However, the possibility of myocardial involvement is raised by the fact that a proportion of neonates with NE have concurrent cardiac dysfunction(135). Martin-Ancel et al showed that 29% of neonates with perinatal asphyxia had cardiac dysfunction consistent with myocardial ischaemia(136). The rate of cardiovascular system involvement in infants with post-asphyxial hypoxic ischaemic encephalopathy is reported to be between 50-78%(137-139). The variation may be due to the difference in criteria used for involvement of cardiac dysfunction in the above studies. Children with epilepsy who are seizure free have been shown to have impaired systolic and diastolic cardiac function using 2-dimensional speckle tracking echocardiography(140). In this case-control study (n=120) there were no differences between the subgroups to explain this subclinical dysfunction and suggest that there may be unknown factors involved. Krishnamoorthy et al prospectively assessed myocardial dysfunction in patients with moderate to severe traumatic brain injury using speckle tracking echocardiography and compared them with age and sex-matched controls (n=30)(141). They have shown that abnormalities of myocardial strain are seen in this population and that the abnormality persists for at least one week. The mechanism underlying this is uncertain but may relate to neuroendocrine dysfunction and dysregulated inflammation(142).

Although it is known that there is acute myocardial dysfunction at the time of the initial insult in some children who develop CP, the long-term myocardial function of these patients is not known. Traditional markers of cardiac function such as ejection fraction/shortening fraction are too crude to recognise potential subtle signs of myocardial
dysfunction and are unable to detect regional wall dysfunction. With the advancement of echocardiography, newer forms of assessing myocardial function are readily available such as deformation imaging using speckle tracking, tissue doppler, tricuspid annular plane systolic excursion, mitral annular plane systolic excursion and fractional area change. These more sophisticated echocardiography techniques may be able to detect early cardiac dysfunction helping to guide management of these complex patients. There is a paucity of research in follow-up of cardiovascular dysfunction throughout childhood in cerebral palsy. Echocardiographic assessment of myocardial strain combined with serum biomarkers may allow more accurate evaluation of cardiac dysfunction in later childhood. Troponin T and N-terminal pro b-type natriuretic peptide (NT-pro-BNP) have been shown to be useful markers of cardiac dysfunction in other populations, such as term neonates with perinatal asphyxia, children with chronic kidney disease and children with leukaemia(143-145). A prospective study of infants with perinatal asphyxia (n=54) showed that Troponin T is elevated in those with abnormal MRI findings and that Troponin T correlates with developmental outcomes at 2 years of age(146). Further research is required to establish whether initial abnormal Troponin T and BNP results correlate with subtle myocardial dysfunction parameters in later childhood.

1.2.7 Renal and urinary tract dysfunction

Renal and urinary tract dysfunction is multifactorial in the population of children with CP. Lower urinary tract dysfunction and renal dysfunction may be linked. Lower urinary tract dysfunction is more common in those with CP, affecting, on average 55.5% of this population, urinary incontinence being the most common symptom(147). Lower urinary tract symptoms (LUTS) are known to have a significant impact on quality of life in children and their families(148, 149). Urinary incontinence is more common in children and adults with CP(150-152). Children with CP achieve continence later than their typically developing peers(152, 153) and experience more daytime incontinence, nocturnal enuresis, or a combination of both(152). Abnormal urodynamic studies have been reported in 85% of children with CP referred for urological assessment (n=27) in the following categories: spastic diplegia (n=10), spastic quadriplegia (n=7), spastic hemiplegia (n=5), dystonic quadriplegia (n=3), dystonic diplegia (n=1), spastic monoplegia (n=1) and intellectual disability (n=15)(154). The commonest presenting symptom was incontinence (74%) followed by frequency (56%) and urgency (37%)(154). Disorders of urinary storage, most frequently reduced bladder capacity, detrusor overactivity and increased post-void residual volumes, are more common than disorders of voiding (e.g. urinary hesitancy and frequency)(154-156). Greater degrees of motor impairment as evidenced by the GMFCS are associated with a greater frequency of lower urinary tract symptoms and urodynamic abnormalities(31). GMFCS and intellectual ability
have both been associated with attainment of continence(152, 157) although agreement on the influence of intellectual ability on continence in CP is not universal(158). Reid et al advocated for early investigation of urological symptoms with urodynamic studies in children with CP as a way to rationalise treatment, noting that in many cases incontinence can be improved or cured(154).

Bladder dysfunction such as interrupted voiding, hesitancy and urinary retention, but not urinary incontinence, have been linked with upper urinary tract deterioration in children with CP(159). Further urological evaluation may thus be warranted in these children. Notably, obstructive voiding complaints are more common in adults with CP than children and may represent progression over time(160). Half of adults with CP had small, high pressure bladders, putting them at risk for upper urinary tract deterioration (n=49, mean age=31 years, 55% male, 98% GMFCS III-V), thus emphasising the need for ongoing monitoring of people with CP and urinary tract symptoms(160). That said, this paper describes the quarter of patients with CP who underwent urodynamic studies according to the centre’s criteria for investigation, which includes recurrent UTI, progressive urinary incontinence, hydronephrosis, urinary retention and progressive LUTS. Therefore, there may be an element of selection bias which skews the results.

Urinary tract infections are more common in children with CP(153, 161) occurring in approximately 20%(162). Children with CP have an increased incidence of constipation and faecal incontinence(152, 163, 164), both risk factors for the development of UTI(165, 166). Recurrent febrile urinary tract infections have been linked with upper urinary tract deterioration in children with CP and should prompt further urological evaluation. Congenital urinary tract abnormalities have not been found to be more common in children with CP(167).

A significant number of children with CP post NE may develop renal dysfunction later in life. Up to 72% of infants who have a 5-minute Apgar score of ≤ 6 show signs of renal compromise(168, 169). Renal involvement in the neonatal period is also associated with neurological severity and outcome(170-173). An episode of acute kidney injury (AKI) in the neonatal period is correlated with an increased risk of developing chronic kidney disease (CKD) in later life (168, 174, 175). Therefore, any children who have had AKI because of perinatal asphyxia, regardless of severity, require regular renal function and blood pressure monitoring.

In a large retrospective cohort study, adults with neurodevelopmental disabilities (n=33,561) had a greater incidence rate of CKD than adults without neurodevelopmental disabilities (n=6.5million). This increased risk was maintained even when adjusted for demographics, diabetes, hypertension, and cardiovascular disease. Adults with neurodevelopmental disabilities, throughout all subgroups, were also more likely to have advanced CKD. The incidence rate ratio of advanced CKD for those with CP compared to
adults without NDDs was 1.83 (95% CI=1.61-2.07) (176). This highlights that children with CP have a greater risk of developing CKD and renal function monitoring including minimising nephrotoxic drugs should be considered.

One of the most widely used methods of monitoring renal function is to measure serum creatinine but it does not rise until there is significant renal dysfunction. In addition, children with non-ambulatory CP have been shown to have lower serum creatinine concentrations at baseline(177). This likely represents a reduction in muscle mass and bone mineral density which is evident in children with CP(178-180) This may present some difficulty in monitoring for CKD as these children may have falsely reassuring creatinine levels, compounded by the fact that early CKD is often silent. In the outpatient setting, markers such as blood pressure, along with urinary protein can be useful for monitoring renal function.

Novel biomarkers such as Neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, Interleukin-6 (IL-6) and Fibroblast Growth Factor (FGF-23) can improve the early diagnosis of AKI and CKD (181-185), predict clinical outcome (186, 187) and help quantitation of severity in CKD(188). One or more of these or other novel biomarkers may prove useful for monitoring of renal function in children and adults with CP but this has not, as yet, been assessed in this population.

1.2.8 Gastrointestinal dysfunction

There is a high prevalence of gastrointestinal disorders in children with cerebral palsy including dysphagia, gastroesophageal reflux disease (GORD) and chronic constipation (189). Dysphagia is related to inefficiencies in the oral preparation, oropharyngeal and oesophageal stages of swallowing. It is a common problem in children with CP, occurring in 43% (190), and can be caused by oromotor dysfunction, oral sensory impairment, abnormal neurological development and oesophageal dysmotility. GORD in these children can make dysphagia worse(32). Clinically significant oral motor dysfunction was demonstrated in a further study in 90% of children with CP(191). Complications of dysphagia in this population include recurrent lower respiratory tract infections and chronic aspiration, as discussed previously, as well as malnourishment, with an association between dysphagia and poor weight gain(32).

The issue of drooling is inextricably linked with dysphagia and GORD and is defined as "the unintentional loss of saliva and contents from the mouth"(192). It occurs in between 37.4% and 58%(193) of children with cerebral palsy and is subdivided into anterior and posterior types(194). Anterior drooling occurs when saliva leaks from the mouth and posterior drooling is where the saliva spills into the oropharynx and hypopharynx(194). Children with cerebral palsy do not have excessive salivation, but an oromotor muscle incoordination and sensory perception issues(195). Severe anterior
drooling can lead to social isolation, damp clothing, irritated facial skin, interference with speech, damage to books, communication aids and computers(196, 197) Posterior drooling can lead to aspiration which may result in pneumonia.

Sialorrhoea can be exacerbated by GORD in children with CP through the oesophagogastric reflex. Treatment of children with CP, Sialorrhoea and gastrooesophageal reflux with antireflux medication has been shown to reduce drooling in a double-blind, placebo-controlled, crossover trial (n=9)(198).

A multidisciplinary approach to the management of drooling is advisable (199, 200). The team can include neurologists, otolaryngologists, paediatricians, plastic surgeons, speech and language therapists, psychologists, physiotherapists, nurses, and teachers. The treatment options vary from less invasive behavioural and non-medical to pharmacological interventions to more invasive surgical management usually reserved for children over 4 years of age.

The two main medical treatments are anti-cholinergic agents and intraglandular botulinum toxin injections to the submandibular and/or parotid glands. The anti-cholinergic drugs most used are atropine, benztropine, glycopyrrolate bromide and scopolamine. Pharmacological interventions cannot selectively block stimulation of the salivary glands and as a result unwanted side effects can occur for example, constipation, blurred vision, behaviour disturbance, urinary retention and thickening of secretions(201).

Botulinum Toxin Injections into the submandibular glands and/or parotid glands under ultrasound guidance is often used if there is an inadequate response to the anticholinergic drugs. The main drawback is that they are only effective for about 6 months, need to be repeated and responsiveness can diminish over time. Parents should be advised of the potential for adverse effects such as xerostomia and dysphagia. (194) There is insufficient evidence on the efficacy of many of these pharmacological treatments in Sialorrhoea (193), however, benztropine and Botulinum Toxin injections have been shown to reduce drooling in a recent meta-analysis(202).

Surgery is usually reserved for children that have failed all the less invasive treatments discussed. Submandibular or parotid duct ligation as well as submandibular gland excision are not without significant complications, although rare. For example, salivary stones, scarring, facial and hypoglossal nerve damage(203, 204) can occur. Submandibular gland duct relocation is useful for anterior drooling(205) but contraindicated for posterior drooling.

Symptoms suggestive of GOR are seen in 77% of children with CP (n=58), of whom 90% were subsequently proven to have GORD by pH monitoring and endoscopic evidence of oesophagitis(189). The increased risk of GORD in children with CP is due to poor posture, scoliosis, increased intra-abdominal pressure from spasticity or medication side effects. These side effects include those of anticonvulsants which may increase
nausea, vomiting and dysphagia, and, in turn, worsen the severity of GORD(206). Prospective analysis of 101 children with NI demonstrated that early onset of NI, mitochondrial disorders and EEG abnormalities were significant risk factors for GOR(207). In a cohort of children with CP and GORD (n=18), significantly prolonged gastric emptying and abnormal oesophageal motility was demonstrated on manometry (p<0.01), suggesting that GI motility dysfunction contributes to GOR and oesophagitis in this group(189).

The diagnosis of GOR in some children with CP may be challenging because of difficulties with communication. GOR has been found in 91% of children with CP who have regurgitation, vomiting, recurrent abdominal pain, haematemesis and/or pulmonary aspiration(189). However, in many cases these symptoms may not be present. Considerable agitation and autoaggressive behaviour can be a marker for pathological GOR and therefore, should be excluded when parents report these behaviours(208).

The North American and European Society for Paediatric Gastroenterology Hepatology and Nutrition (NASPGHAN, ESPGHAN) have published guidelines on the treatment of GORD, based on the available evidence(209). Lifestyle factors such as raising the head of the bed and avoiding exposure to smoke may be helpful in treating GOR. Thickening of feeds can help to reduce overt regurgitation and vomiting(209). The mainstays of pharmacological management are histamine receptor antagonists (H2RA) and proton pump inhibitors (PPI). It is recommended that PPIs are used as first line pharmacologic treatment of GORD, with use of H2RAs if PPIs are not available or contra-indicated(209). Baclofen is an alternative which may be considered prior to surgery if other medical treatments have failed(209). Surgical interventions, such as fundoplication, can be considered in children with GORD and neurological impairment if optimal medical therapy has been unsuccessful, with total oesophagogastric dissociation reserved as a rescue procedure for those in whom fundoplication has failed(209).

Children with more severe CP are at greater risk of poor nutritional status.(210) It is estimated that up to 46% of children with CP can be classified as undernourished (211), however, it is only documented in 7.9% of children with CP admitted to hospital, suggesting significant under recognition(212). Dysphagia, GORD and constipation were significantly associated with malnourishment (212). ESPGHAN has published detailed recommendations for monitoring and managing nutritional status in children with NI, including biannual monitoring of anthropometric measurements and annual measurement of micronutrients. Nutritional optimisation may be beneficial in improving the functional status of children with CP(213). In a prospective study (n=14) where children with CP and malnourishment completed a 6 month nutritional rehabilitation programme, 64% had an improvements in their “Gross Motor Function Measure” scores(214).
Constipation is also a substantial problem in children with neurodevelopmental disabilities (215) affecting almost ¾ of children with CP (189). This is mainly due to prolonged transit time, particularly in the more proximal segments of the colon (189). Treatment of constipation for children with NI is not significantly different than their typically developing peers apart from avoidance of polyethylene glycol and liquid paraffin in those with significant risk of pulmonary aspiration. Additionally, increased fluid and fibre intake can be considered in this population (216).

Children with NI are at significant risk of acute and chronic pain with the abdomen accounting for a significant proportion (11-32%) of these symptoms (217, 218). In a cross-sectional study involving children with CP aged 5-18 years (n=288), 67.1% had acute pain and 31.4% had chronic pain with risk factors including dyskinesia, bilateral involvement and GMFCS IV-V (219) and, of those with acute pain, 42% also reported chronic pain (219). Abdominal pain may occur because of nociceptive pain sources such as appendicitis, pancreatitis, or cholecystitis, all of which may be more difficult to diagnose in children with CP, particularly if they are non-verbal. Children with CP are also at increased risk of pain as a direct result of neurological dysfunction, including central neuropathic pain and visceral hyperalgesia (217, 220). This is particularly seen with lesions in the thalamus and spinothalamic tract. In these situations, normal or minimally noxious stimuli can result in significant pain (221). This means that routine activities, such as feeding, which inevitably produce gut distension, may provoke considerable pain. Non-pharmacological approaches to treatment such as reduction in feed volumes, venting of feeding tubes or alterations in feed timing may be useful. Management of pain, including abdominal pain, in these children should initially begin with a thorough assessment for any nociceptive sources of pain. If no obvious source can be identified a trial of empiric medical treatment with gabapentin has been suggested, with escalation to tricyclic antidepressants and methadone considered the following steps (222). In two retrospective studies (n=22 and n=42), gabapentinoids have been shown to reduce pain behaviours (223, 224). Prospective trials to confirm efficacy in this cohort would be useful. Above all, a systematic and thorough approach to assessing and managing pain in these children is required.

Gut and intestinal failure is increasingly being recognised in children with neurodisabling conditions (225). This is not surprising given the sizeable interface between the central and enteric nervous system. However, there is a significant paucity of research in this area which should be addressed in the future.

1.2.9 Haematological

There is evidence to suggest that cerebral infarction secondary to pre or perinatal cerebral occlusion occurs in 13-37% children with hemiplegic CP and the infarction may
occur due to a thrombophilia (226-228). Gunther et al (227) studied 91 cases of neonatal arterial-ischaemic stroke and found that 68% had at least one pro-thrombotic tendency. However it is important to note that 24% of the 182 controls also had an abnormality. Mercuri et al noted that the factor V Leiden mutation in particular may be associated with poorer outcomes in neonatal stroke (228). Coagulopathies and thrombophilias have been reported to be associated with neonatal stroke include antiphospholipid antibodies (229), anti-cardiolipin antibodies (230), increased lipoprotein (231), protein C resistance (231) and protein C and S deficiencies (226). The American Academy of Neurology and Child Neurological Society have recommended that diagnostic testing for a coagulation disorder should be considered in children with hemiplegic CP although the level of evidence was relatively low (226). The NICE guidelines for assessment of cerebral palsy (38) make no recommendation for routine testing for coagulation or thromboembolic disorders in children with CP.

The coagulation and inflammatory systems overlap and interact. Activated coagulation factors are proinflammatory and in turn inflammation can promote coagulation (232). Elevated inflammatory markers and coagulation factors coexist in neonates who subsequently develop CP (232). In children with NE, a risk factor for CP, it is known that coagulation parameters are strong predictors of outcome (233). Antenatally, it has been shown that children who subsequently develop CP are more likely to have had 2 or more placental lesions of thrombosis or inflammation (234) and that foetal thrombotic vasculopathy is significantly associated with NE (235). It is likely, therefore, that the interplay between coagulation and inflammation contribute to white matter damage and multi-organ dysfunction (236) and are thus important in the aetiology of CP (232).

Beyond the neonatal period, children with CP have prothrombin times (PT) and activated partial thromboplastin times (APTT) which are within the normal ranges but which are significantly longer than controls (237). They also have significantly reduced calcium and magnesium levels, both important cofactors for coagulation (237). Brenn et al examined bleeding in children with severe spastic quadriplegia during posterior spinal fusion surgery compared with controls (n=34) and found that children with CP have significantly more bleeding and that bleeding occurs earlier in the course of surgery (237). Kannan et al also reported significantly more blood loss during spinal surgery in children with neuromuscular scoliosis compared to those with idiopathic scoliosis (n=25) and found reduced factor VII activity (238). Children with CP are also often taking medication which can effect coagulation, as reported in several case reports, such as valproate (239, 240), carbamazepine (241) and azithromycin (242). Therefore, consideration of coagulopathy and thrombophilia in children with CP is important, particularly prior to surgery, including the administration of prophylactic tranexamic acid and early use of blood products (243).
There is a high rate of nutritional deficiency amongst children with CP (244-246), which may correlate with anaemia. However, evidence regarding anaemia in children with CP is sparse. In institutionalised children and young people (n= 108, age 8-29 years) with cerebral palsy, iron-deficiency was reported in 38% and anaemia was found in 33.3% of participants. Both iron-deficiency and anaemia were significantly increased in those who had liquid diets compared to normal diets (95.6% vs 22.3% and 87% vs 18.8% respectively) (247). In another case series (n=30) iron-deficiency anaemia was found in 4 (13.3%) children with neuromotor disabilities (248). In comparison, rates of iron-deficiency anaemia in both low and middle-income children in the United States of America have been reported to range between 2-3% (249, 250). This is significant because iron-deficiency, even without anaemia, is known to affect neurodevelopment including motor, cognitive and social-emotional function (251). In children with spastic CP, it has been reported that iron deficiency anaemia has a negative effect on functional ability and muscle strength (252). Further research is needed to quantify the extent of iron deficiency and anaemia in children with CP to ensure adequate treatment if necessary.

1.2.10 Inflammation and Infection

NE accounts for around 24% of all cases of CP in term infants (21, 22). Infants with NE have been reported to have persistent inflammatory response over the first week of life, with higher neutrophil, monocyte and Toll-like receptor-4 expression, correlating with the degree of brain injury (253, 254). High concentration of Interleukin (IL)-1, 8, 9 and tumour necrosis factor (TNF)-α were demonstrated in neonatal blood from children with Cerebral Palsy in comparison with control children (255). One of the most important underlying pathophysiological mechanisms leading to CP includes intra-amniotic inflammation and infection eliciting an inflammatory response and damage to the developing brain. Foetal inflammatory response syndrome (FIRS) is a severe form of intra-amniotic infection or inflammation. It stimulates the activation of innate immune system of the foetus, similar to that seen in adult inflammatory response syndrome. FIRS can lead to multi-organ dysfunction and causes neurological, renal and haematological abnormalities (256).

Perinatal inflammation is also associated with many neuropsychiatric and neuropsychological disorders and it is suggested that inflammation has long term consequences on the brain during childhood (257). Children post-NE have been shown to have persistent inflammatory responses at school age (127). The injury processes can persist for months and years and a tertiary mechanism of damage, which includes inflammation and epigenetic changes, has been proposed (257). Dysregulated immune function is also found in school age children with CP who had brain injury in the neonatal period (126). School-age, preterm children with CP secondary to PVL had significantly
higher levels of TNF-α in peripheral blood mononuclear cells (PBMC’s) after lipopolysaccharide (LPS) stimulation compared to control school-age preterm children with normal neurodevelopment.

Infections are a significant contributor to childhood death, particularly in those with underlying conditions. CP was the most common underlying condition associated with death due to infection in the 1-4 year age category(258). Pneumonia in particular is an important cause of morbidity and mortality in children with CP, especially among those with severe spastic quadriplegia, epilepsy and intellectual disability (259). An observational data-linkage study of a developmental anomaly registry with a national death index revealed that, of those with available causes of death, 58.6% were attributable to respiratory causes, of whom 49% died of pneumonia at a mean age of 14.6 years(260). Non-respiratory infections accounted for a further 5% of deaths at a mean age of 16.6 years.

Children with CP also appear to be at increased risk of infective complications following surgery. In a retrospective analysis of 1,250 children with CP who underwent appendectomy had a significantly greater risk of developing sepsis/organ failure (adjusted OR=2.47; p=0.13), operation-related infections (adjusted OR=2.65; p=0.001), pneumonia (adjusted OR4.26; p<0.001), and urinary tract infection (adjusted OR=5.19; p<0.001)(261). The mechanisms underlying this increased risk are unknown and likely multifactorial.

Vaccination is one of the most successful ways of preventing infections. Children with CP should follow the vaccination schedule in the country in which they live. However, additional vaccinations may be recommended to provide them with added protection given their associated co-morbidities. Children with CP have a 2-3 times higher risk of incomplete or delayed immunisation(262) A retrospective review of data from children less than 7 years of age included in the Victorian CP register (n=476) found that 19.2% were overdue at least one vaccine(262). It has also been reported that children with moderate to severe motor impairment are less likely to have received all of their vaccinations than those with less severe impairment (n=98; p<0.05)(263).

Children with neurological disorders have been acknowledged as a population which is at increased risk of serious complications of influenza(264). Despite this only 74% of paediatricians in the United States recognised CP as a high-risk condition (n=412)(265). This survey-based study also found that 50% of children with a neurologic or neurodevelopmental disorder (n=1143) were already vaccinated or their parents planned for vaccination against influenza(265) and programmes to improve the uptake rate are required.

A large population-based, case-control study in Denmark (n=1665) shows a higher risk of invasive pneumococcal disease amongst children with several chronic conditions.
The adjusted risk ratio for children with a neurological disorder compared with children without such a disorder was 3.0 (95% confidence interval=2.1-4.3)(266). There may be an argument for providing these children with extended-coverage polyvalent pneumococcal vaccines but this requires further research.

Recent research has shown that inflammatory responses in children with CP are altered. Further research may help to clarify the role of inflammation as a tertiary cause of neurological injury with the potential to develop therapeutic targets. There may also be a significant impact on immune function and further work is needed to optimise vaccination programs for children with CP and improve infection-associated morbidity and mortality.

### 1.2.11 Metabolic

Inborn errors of metabolism (IEM) can present with CP like symptoms. A systematic review by Leach et al. identified almost 70 IEMs belonging to 13 different biochemical categories, that closely mimic cerebral palsy (CP). While these only account for 0.1-0.2% of CP cases, early diagnosis is essential to prevent organ damage(267). Metabolic investigations should be considered where the clinical history is consistent with a metabolic disorder. These features may include chronic progression, neurodevelopmental regression or non-central nervous system (CNS) organ involvement(268). Other signs include absent history of perinatal injury, and pattern of disease inheritance; so called “familial CP” which can be elicited by obtaining a thorough family history and/or presence of involuntary movements, ataxia, muscle atrophy, oculomotor abnormalities or sensory loss(267, 269, 270). In children with CP and normal magnetic resonance imaging (MRI), further testing for metabolic and/or genetic conditions has been recommended(271, 272). The presence of abnormal MRI findings which are unexpected or inconsistent with the suspected aetiology should also warrant metabolic investigations(271). An example of this are globus pallidus abnormalities which can be indicative of the rare neurometabolic disorder, neurodegeneration with brain iron accumulation (NIBA) with pantothenate kinase-associated neurodegeneration (PKAN) the most frequent subtype identified(273).

Individualised investigations for IEMs can be planned according to the individual clinical picture i.e. history, examination and any neuroimaging. These investigations may include plasma, urine or cerebrospinal amino acids, a plasma acylcarnitine profile and urine organic acids, mucopolysaccharides and oligosaccharides(269, 274). Targeted biomarker analysis or single gene analysis are useful where the phenotype and investigative results highly correlate with a specific metabolic condition. For example a clinical picture of significant dystonia, macrocephaly and MRI revealing widened Sylvian fissures and basal ganglia abnormalities may be confirmed as glutaric aciduria type 1 with urine organic acid analysis and genetic study of the glutaryl CoA dehydrogenase (GCDH)
However due to the overlapping and, in certain instances, unspecific phenotypes of IEMs, next generation and whole exome sequencing has proven useful in non-specific findings where no distinct aetiology is suspected (267, 269). Due to the sheer number of IEMs and the generalised diagnostic approach currently used, Metabolomics has emerged as an innovative method of improving diagnostic efficiency of IEMs as there are numerous possible diagnoses and it may be potentially used as a tool in precision medicine(275, 276). It should be used in conjunction with next generation and exome sequencing to help clarify the pathogenicity of genetic variants.

Mitochondrial disorders are neurometabolic diseases which can also present with CP like symptoms(267, 270). They are characterised by dysfunctional energy production and typically manifest as multi-organ dysfunction, often with neurological impairment and can be similar to CP(269). Mitochondria are postulated to play a significant role in hypoxic-ischaemic events which may lead to CP and mitochondrial targets are now being explored as a potential future therapeutic intervention as an alternative to therapeutic hypothermia in those with perinatal brain injury. These targets include protecting from mitochondrial permeabilization, directly targeting mitochondrial downstream apoptotic pathways and indirect protection and preservation of mitochondrial function(277).

It is important to be mindful of potential metabolic diagnoses when seeing children with CP as they can have significant implications, the most significant of which is to prevent deterioration and improve clinical outcomes.

1.2.12 Genetic

An increasing number of studies implicate genetics in the aetiology of cerebral palsy (CP). A genetic component to cerebral palsy may prompt the clinician to be particularly vigilant for specific multi-organ abnormalities. Several non-metabolic single gene disorders can present as CP. Many single gene mutations linked with CP have been identified using whole exome sequencing, including mutations in KCNC3, KANK1, AP4MI, GAD1 and ADD3 with different mutations linked to the development of different subtypes of CP(278-281). For example, mutations in KCNC3 have been found in some individuals diagnosed with ataxic CP(278) and heterozygous deletions of KANK1 are associated with early motor delay and hypotonia, progressing to spastic quadriplegia and intellectual disability(282). Each of these single gene disorders are individually rare but together may account for a significant number of cases of CP. They are important to recognise as a genetic diagnosis can help with prognosis, monitoring and family counselling.

It is clear there is no single “CP gene” but there is increasing recognition of a genetic element to CP in a large number of cases. Despite improvements in obstetric, prenatal and perinatal care, there has been little change in the incidence of CP in term neonates, which may be due to an underlying genetic pathophysiology(279, 282). In most
studies the prevalence of CP appears to be higher in the male population compared to the female population and it has been suggested that recessive X-linked chromosome variants may contribute to this difference(279). Higher rates of CP have also been reported in monozygotic twins and consanguineous families(280). Furthermore, the presence of coexisting congenital anomalies is significantly higher in the CP population compared to their healthy counterparts with rates of 11-32% and 1-2% respectively(279, 280).

A significant number of candidate CP genes have also been identified alongside other genetic polymorphisms which have been proposed to contribute to the aetiology of CP including copy number variants and single nucleotide polymorphisms(280). De novo and inherited genetic variants may account for up to 30% of CP cases and have been postulated to, in some cases, directly cause CP, while in others merely contribute to CP susceptibility(279, 283). For example, a large Australian case-control study of children with CP (n=443) found that polymorphisms in Toll Like Receptor-4 (TLR-4) reduced the risk of developing CP, while polymorphisms in IL-6 and IL-8 may increase susceptibility to CP in the presence of viral exposure(284).

Finally, epigenetic modifications are gaining increasing recognition as contributing to tertiary brain damage following an initial insult(285). Epigenetics refers to any process by which gene activity is altered without changing the DNA sequence, including methylation and histone, acetylation or micro-RNA modifications(286, 287). It is thought that early life exposures can modify the epigenome and provide a link to neurodevelopmental outcome(288, 289). Epigenome-wide analysis of 15 monoyzotic twins discordant for CP showed regional differences in DNA methylation associated with development of CP(290). Gene loci involved were associated with hypoxia, inflammation and cell adhesion. Further research with larger sample sizes would be useful to confirm these findings. Differences in methylation were also noted to be significantly different between adolescents with CP and controls (n=32, p<0.05)(291). In the future, the epigenome may have potential as a therapeutic target to improve neurodevelopmental outcomes in CP.

The vast genetic heterogeneity underlying CP emphasises the complexity of the contribution of genetics to the aetiology and development of CP and points towards a multifactorial aetiology of CP with interaction between genetics and the environment(280, 292). While genetic causation should not change the clinical diagnosis of CP, inclusion as a subclassification by aetiology may allow for more targeted therapy in these cases(282, 283).
1.2.13 Endocrine

All children with disability are at risk for some form of endocrine dysfunction due to the disruption of endocrine feedback secondary to abnormalities in muscle, bone, or brain mechanisms. Regular assessment from an endocrine perspective should be considered with the emphasis on growth, pubertal status, and bone health. Knowing the underlying aetiology of cerebral palsy can focus endocrine evaluation but is not essential.

All children with cerebral palsy should have formal measurement of body proportions at least twice per year to guide nutrition and to identify growth anomalies. Assessing length or height in proportion to weight is important to ascertain whether short stature is due to nutritional deficiency or a hormone imbalance. Nutritional deficiency is more likely to result in delayed puberty. Exogenous obesity can drive growth in childhood but following puberty can result in obesity in an immobile child causing long term implications for respiratory function and sleep apnoea. Children with midline defects will have hypothalamic pituitary axis dysfunction which can result in growth hormone deficiency impacting on tone, mood, and glycaemic control as well as stature. Studies have indicated that circulating GH secretion profiles are lower in children with cerebral palsy compared to controls (293, 294). Hypoglycaemia can occur as a consequence of metabolic stress in the newborn period, growth, or cortisol hormone deficiency. Deranged glucose metabolism may only become apparent at times of stress and illness and in children with spasticity and seizure disorders could be missed.

CP is the most common childhood condition associated with osteoporosis, and children with CP frequently sustain fractures with minor trauma (295). Any child or adolescent with immobilisation, nutritional deficiency (especially impacting vitamin D or calcium metabolism) or delays in pubertal onset will be impacted by a reduction in bone mass accrual. Apparent reduction in bone density may also be artefactual if not adjusted for height. Growth hormone has been shown to be positively associated with surrogate markers of bone turnover in puberty (296) suggesting that this could be a factor in the attainment of bone density due to the lower circulating levels of growth hormone (294). In a systematic review of children with severe cerebral palsy, significant determinants of low BMD were limited ambulation, feeding difficulties, previous fracture, anticonvulsant use, and lower fat mass (297). The International Society of Clinical Densitometry has identified the importance of correct bone mineral density assessment in cerebral palsy and has issued official positions on both the definition of osteoporosis in this condition (298) and the appropriate site of BMD assessment using bone densitometry (299). If a child with CP is considered at risk of osteoporosis, bone densitometry (DXA) scans should be performed to assess baseline at 6 years of age, and repeated every one to two years depending on individual risk factors (295). In treating children with CP and osteoporosis there is probable evidence for bisphosphonates, possible evidence for vitamin D and
calcium supplementation and insufficient evidence for weight bearing activities as effective interventions to improve BMD (300, 301). Bisphosphonates have been shown to improve pain on manipulation in children with CP and osteoporosis. (302)

Puberty also plays a role in the development of bone health. Clinical assessment of puberty with Tanner staging is therefore recommended (303). Mean age of breast development in girls with CP is similar but with wider range of onset, while Menarche occurs later in Caucasian girls with CP (304). Early adrenarche is seen in cerebral palsy but more commonly in those with hydrocephalus or associated epilepsy. This is not indicative of central precocious puberty and can progress slowly over many years before true pubertal onset (305). Many children and adolescents with cerebral palsy can experience normal pubertal progression and menses (306).

1.2.14 Orthopaedic

Scoliosis and hip dislocation are common problems in children with CP. The overall incidence of scoliosis in CP of 20-25% (307), with a risk of up to 90% for spinal deformities in patients with GMFCS level V (308). Scoliosis is related to poor truncal tone and muscle weakness (309) and predictors for scoliosis include GMFCS IV and V, epilepsy, and limited knee extension (310). The scoliosis pattern in children with GMFCS IV is usually a single long C-shaped curve that is most often kyphotic, but sometimes lordotic. In ambulatory patients with less motor involvement the pattern of deformity may be similar to that in idiopathic scoliosis with both thoracic and lumbar components (309, 311). Progression of scoliosis is usually gradual, but onset of puberty, deterioration in neurological function or prolonged time spent in a wheelchair can lead to a more rapid progression (309). Progressive scoliosis causes difficulties with daily care and mobilisation, can lead to pain, cardiac and pulmonary complications, altered seizure thresholds and skin compromise (312).

Hip dislocation develops in 15-20% of cases (313). There is almost a direct link between higher levels of GMFCS and risk of hip subluxation. Risk of developing a hip migration index (MI) >30% is approximately 30%, 50% and 80% at GMFCS III, IV and V respectively (314). Rate of progression of the MI increases from almost 2% at GMFCS III to 12% at GMFCS V (314). Hip dislocation develops because of contractures and spasticity of adductors, hamstrings, and hip flexors (315). The combination of this spasticity and reduction in weight bearing lead to acetabular dysplasia (316). Hip surveillance is considered essential in reducing hip dislocation and need for surgery (317). Hippotheraphy may lead to improved symmetry and stability although grade of evidence is low (317). Splints may reduce rate of dislocation but are ineffective at preventing hip dislocation (317). There is insufficient evidence as to whether therapy with botulinum toxin can prevent hip dislocation (318).
Both aforementioned orthopaedic complications of CP may be linked. Hip dislocation or windswept deformity of the hip may cause pelvic obliquity and trigger scoliosis, while scoliosis itself can lead to pelvic obliquity and thus increase the risk of hip dislocation especially on the high side (313). Early identification of these issues of scoliosis and hip dislocation are important for improved surgical outcomes, although there are multiple prerequisites before consideration of surgery, such as Cobb angle > 40 to 50 degrees which is progressive and interfering with sitting, age and medical comorbidities (309).

The upper limb may also develop significant complications in CP. Upper limb contractures developed in 34% of a population-based sample of children with CP (n=771). These contractures began at preschool age and the best predictor was high scores on the Manual Ability Classification System (319). The level of evidence for interventions relating to the upper limb is much lower than that for the lower limbs. Further high-quality studies in this area are required.

1.2.15 Summary of MOD in CP

Multi-organ dysfunction in cerebral palsy to date has not been quantified. Better insight into multi organ dysfunction in children with CP can be gained by using novel biomarkers and new diagnostic tools. In this section, we focused on various long-term issues associated with each organ system and identified parameters and novel biomarkers for monitoring MOD. In the future, with further study, biomarkers including serum and urinary NGAL, cystatin C and IL-6 show good ability to predict AKI and may be useful in long-term follow up. Cardiology assessment including measurement of serum troponin, pro-NT-BNP, echocardiography using tissue doppler and speckle-tracking may be used to monitor cardiovascular function in children with CP.

In addition, children with CP have altered immune function compared to age-matched controls. Understanding the immune response in these children with CP and exploring systemic inflammation holds promise for future development of immunomodulatory adjunctive therapies. Further research in this can contribute to better prediction of outcome and improved prognosis.

A MOD scoring system may prove useful in allowing advanced clinical planning and follow-up of children with CP throughout the lifespan. CP registries are now in existence throughout the world. The information provided by these registries, as well as the expert collaboration that they promote, in conjunction with a MOD scoring system, might allow for the production of tailored guidelines for follow-up and management of MOD in children with CP. We suggest that follow up of multi-organ function is important to identify and pre-emptively manage potential long-term complications in CP.
There is a significant need for an awareness amongst healthcare professionals of the multi-systemic complications that are associated with CP, particularly in those with greater levels of impairment. That many of these children communicate non-verbally compounds the difficulties that the physician faces in understanding the source of many of their symptoms. The cause of symptoms such as pain, behavioural or sleep disturbance may be multi-factorial and present substantial diagnostic challenges. A stepwise, logical, multi-system approach, with support from a multi-disciplinary team, is required to ensure that the best care is provided to these children and their families.
Table 1.1. Summary of multi-organ involvement, management and potential future directions for research in Cerebral Palsy (CP).

<table>
<thead>
<tr>
<th>Possible considerations for care</th>
<th>Treatment and potential future directions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neurological</strong></td>
<td>Consider genetic and metabolic investigations if brain malformation, unclear aetiology, or findings atypical (320)</td>
</tr>
<tr>
<td>MRI brain for all (320)</td>
<td>Seizure management does not significantly differ from children without CP. (38)</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>Medical options for spasticity management include diazepam, baclofen (oral or intrathecal) and botulinum toxin (42)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td></td>
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<tr>
<td>Behavioural issues, including inattention and hyperactivity</td>
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</tr>
<tr>
<td>Spasticity</td>
<td></td>
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<tr>
<td><strong>Hearing and Vision</strong></td>
<td>Consider thorough audiological assessment if concerned</td>
</tr>
<tr>
<td>Hearing impairment</td>
<td>Screening questionnaires are available to help with recognition of CVI (48)</td>
</tr>
<tr>
<td>Cortical Visual Impairment (CVI), refractive errors and accommodative dysfunction are common in CP (48, 321)</td>
<td>Early and periodic screening of vision recommended</td>
</tr>
<tr>
<td><strong>Respiratory</strong></td>
<td>Benefit of treating GOR for respiratory reasons unclear. Large prospective trials required</td>
</tr>
<tr>
<td>Significant cause of morbidity and mortality</td>
<td>Mucolytics and physical therapies may help with tenacious secretions (322)</td>
</tr>
<tr>
<td>o Largest cause of premature death in children and young people with CP (53)</td>
<td>Prophylactic antibiotics – randomised controlled trials required</td>
</tr>
<tr>
<td>Dysphagia</td>
<td></td>
</tr>
<tr>
<td>Aspiration</td>
<td></td>
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<tr>
<td>LRTI more common and severe (71)</td>
<td></td>
</tr>
<tr>
<td>Bronchiectasis (74)</td>
<td></td>
</tr>
<tr>
<td>o SNI and GOR most significant risk factors</td>
<td></td>
</tr>
</tbody>
</table>
| Sleep | • Sleep disorders  
  - Association with seizures(89)  
  - DIMS most common subtype(90)  
• Sleep disordered breathing more common in CP(89, 117) | • Screen for sleep disorders using validated tools  
• First-line therapy – sleep hygiene(105, 107)  
• Trial of melatonin where no identifiable cause of sleep disturbance found(38)  
• Adenotonsillectomy first-line treatment for OSA(123)  
• Consider non-invasive ventilation but high failure rate(118) |
| Cardiac | • Adults with CP have 3-fold increase in CVS disorders(124)  
• Altered inflammation and reduced activity are risk factors for endothelial dysfunction and later atherosclerosis(125, 126, 323) | • Long-term follow up studies required to demonstrate predictive value of CIMT and HRV on adult morbidity and mortality  
• Potential for use of serum biomarkers, such as Troponin, and advanced echocardiography to evaluate cardiac dysfunction in CP |
| Renal and Urinary Tract | • Lower urinary tract dysfunction(147)  
  - Urinary incontinence most common  
  - Bladder dysfunction may be linked with upper urinary tract deterioration  
• UTI more common in CP(161)  
• Higher risk of CKD(176) | • Blood pressure and urinary protein currently useful for monitoring renal function  
• Potential for novel biomarkers such as Cystatin-C, NGAL and IL-6 in the future but further research required in this population  
• Minimise nephrotoxic drugs  
• Low Creatinine may not be a reliable method of monitoring for renal dysfunction in CP |
<table>
<thead>
<tr>
<th>Category</th>
<th>Points</th>
<th>Points</th>
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| Gastrointestinal | • Dysphagia  
• Drooling  
• Diagnosis of GOR may be challenging due to communication difficulties  
• Nutritional optimisation associated with better functional status(213)  
• Constipation  
• Increased risk of acute and chronic abdominal pain which may be difficult to diagnose particularly if non-verbal(217, 218) | • Multi-disciplinary approach to management of drooling  
• Anticholinergic agents and intraglandular botulinum toxin are mainstay of medical treatment of drooling(202)  
• Use PPI as first line treatment of GORD with H2RAs as alternative. Consider surgical alternatives if medical treatment fails(209)  
• Annual monitoring of micronutrients(213)  
• Thorough assessment for any nociceptive sources of abdominal pain. If no source evident consider trial of gabapentin(222)  
• Further research required on gut failure in CP |
| Haematological | • Thrombophilias have been associated with neonatal stroke and hemiplegic CP(231)  
• Increased blood loss seen during spinal surgery(237)  
• Iron deficiency has a negative effect on functional ability and muscle strength(251) | • Consider prophylactic tranexamic acid and early use of blood products for surgical interventions(324)  
• Monitor for iron deficiency and anaemia and treat accordingly |
| Inflammation and Infection | • Children post-neonatal encephalopathy have altered inflammatory responses which persist until school age(127)  
• Children with CP are at increased risk of all infections, including respiratory infections, post-operative infective complications and invasive pneumococcal disease(258, 259, 261, 266) | • Further research is required to show whether children with CP have persistent inflammatory and immune dysfunction  
• Follow national immunisation schedule, recommend influenza vaccination and consider extended-coverage polyvalent pneumococcal vaccine |
| Metabolic | • Metabolic disorders may mimic CP | • Consider metabolic testing if progression, developmental regression, atypical history or neuroimaging, positive family history(268) |
### Genetics

- Single gene disorders may produce a CP phenotype (278)
- Genetic polymorphisms may increase susceptibility to developing CP (284)
- Further research will likely expand the number of genetic disorders and polymorphisms known to cause or increase susceptibility to CP
- The epigenome provides promise as a therapeutic target to improve neurodevelopmental outcome in CP

### Endocrine

- Increased risk of growth anomalies secondary to GH deficiency and nutritional deficiency (293)
- Most common childhood condition associated with osteoporosis (295)
- Early adrenarche is seen in CP but often not indicative of true central precocious puberty (305)
- Formal measurement of body proportions at least twice per year
- If concerned re osteoporosis, perform DXA at 6 years and then biennially (295)
- Evidence for treatment with bisphosphonates, vitamin D and calcium in CP but further work required on benefit of weight-bearing (300, 301)
- Regularly assess puberty with Tanner staging (303)

### Orthopaedic

- Scoliosis – associated with poorer gross motor function (308)
- Hip dislocation - associated with poorer gross motor function (313)
- Upper limb contractures
- Hip surveillance - dependent on GMFCS
- Further research on effectiveness of interventions to prevent hip dislocation essential
- Level of evidence for interventions relating to the upper limb is lower and requires further study
1.3 Inflammation and immunity in children with SNI

Children with SNI have multi-organ dysfunction, the extent of which is not currently known. However, it is hypothesised that the immune system, including its delicate pro- and anti-inflammatory balance, is one of the systems involved. Antenatally, the inflammatory response can be particularly injurious to the developing brain (325) and it is proposed that ongoing inflammation may result in tertiary damage to the nervous system (257). Preterm birth, intrauterine infection and inflammation are some of the most important causative factors in the development of CP (326). It is also proposed that inflammation may act as a sensitising agent which may predispose the brain to further injury when exposed to subsequent, minor insults, leading to an “injurious cascade” (327). If an acute inflammatory process fails to resolve and a chronic inflammatory state ensues, this may lead to increased risk of several neurodevelopmental disorders (257). Children with NE, of whom 24% will develop CP, have been shown have persistent systemic inflammatory responses over the first week of life correlating with the degree of neurological injury (253). They also have altered cytokine responses to endotoxin as compared to controls when measured at school-age, with increased interleukin (IL) -2, IL-6, IL-8, tumour necrosis factor β (TNFβ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (127).

Inflammation provides a possible therapeutic target for improving neurological outcomes for these children. The persistence of inflammation in the populations of children mentioned here may mean that the opportunity for intervention extends further beyond the neonatal period than previously anticipated. Furthermore, persistent systemic inflammation may contribute to dysfunction of organs outside of the neurological system, such as the renal, cardiovascular and respiratory systems, with potential for associated morbidity and mortality.

1.4 Wellbeing of children with Severe Neurological Impairment and their Families

Wellbeing is a complex concept which has garnered increasing attention in the last number of years. Most would agree that wellbeing encompasses much more than the absence of mental illness. There is much focus on the “hedonic” tradition of subjective wellbeing which relates to maximising happiness and minimising negative experiences. However, another element of wellbeing is termed “eudaimonic” and argues that wellbeing comes from pursuing ones potential and having meaning and purpose in life. The presence of both is required for a person to “flourish” and to attain the highest level of wellbeing (328).
Children with SNI require much assistance with activities of daily living. This extra assistance is usually provided by families, perhaps with the assistance of local healthcare authorities, depending on jurisdiction, allocation of resources and need. As a result, there may be additional pressures experienced by the parents or guardians of these children, whether they be financial, social or emotional, or a combination of multiple factors. The wellbeing of the family, including siblings, may, therefore, be affected.

There are financial implications to caring for a child with SNI. Caregivers of children with CP have a lower income and are less likely to work full time outside of the home or report working for pay. They are more likely to report caring for their child as their main activity (329). A national survey in the United States (n=42,000) showed that families of children with disabilities face significantly more material hardship than families raising children without disabilities. The same study found that although the risk declines with increasing family income, a significant proportion of middle-class families with a child with a disability suffered from material hardship(330). It is likely that there is a link between poverty and poorer parental health and wellbeing(331). In fact, one of the main factors associated with depression in families caring for children with intellectual and developmental disabilities is lower household income(332).

Families caring for a child with a disability require significantly more resources than the general population to facilitate their social participation (333) which may result in social isolation. The need for support is greater when the child or young person is more severely impaired, incontinent or has fragile health(333). A child’s disability has an impact on everyday life of the family e.g. half of parents reported having to give up their hobbies as a result of raising a child with a disability(334). The social impact of a child’s disability can be influenced by many factors, including societal and self-stigmatisation(335, 336) and the associated social isolation is consistently reported to have a negative effect on parental health and wellbeing(337).

Parents of children with disabilities have poorer psychological and physical health (329) and mothers of these children can find caregiving “relentless and challenging”(338). Their perception of the severity of their child’s disability is associated with their perception of reduced social support and higher levels of anxiety and depression.(339). A third of parents caring for children with intellectual and developmental disabilities have moderate depression and a third have moderate anxiety. This is compared with 7% and 14% respectively in parents of children without an intellectual or developmental disability(332). High or very high psychological distress is reported in 48% and suicidality is reported in 22% of mothers of a child with a disability(340). Only 58% of mothers attempted to access psychological support despite 75% reporting a need(340).

The experience of fathers of children with a disability is significantly underrepresented in the literature and much of the literature reporting on parental
experiences is skewed towards mothers. A systematic review and meta-analysis by Dunn et al found that fathers of children with intellectual disabilities were less likely than mothers to experience poor mental health (341). Most of the studies that were reviewed reported poorer mental health in fathers of children with disabilities than the general population. However, based on a small number of studies, the pooled results did not show a statistically significant increase in depression (341). These results are supported by a population based study in the UK which showed that fathers of children with intellectual disability had slightly poorer wellbeing and general health but that issues such as employment and income were more important determinants than their child’s disability (342). The differences between mothers and fathers may be due to traditional societal roles, with fathers less involved in caregiving and more likely to be in paid employment, thus, reducing social isolation and life dissatisfaction (341). There may also be influence from the fact that men are more inhibited in their emotional expressiveness and help-seeking behaviours with regards to mental health (343).

The sibling relationship is unique and has a significant bearing on a child’s development. Siblings of children with disabilities are known to have reduced indicators of wellbeing in some, but not all domains of wellbeing and these differences were noted to be small (344). Siblings of children with special healthcare needs (CShCN) or neurodevelopmental disability are at heightened risk of emotional and behavioural problems, peer relationship difficulties and functional impairment (345-347). Male siblings appear to have more emotional and behavioural problems than female siblings (347). Much of the literature focuses on siblings of children with intellectual disability but the findings from these studies do not necessarily translate to the experiences of children in families where there is a child with physical disabilities, who face distinct challenges. Severity of disability and extent of medical co-morbidities also need to be taken into account.

At this point, it is important to mention the undeniably positive impact that many children with disability have on their families and bring great joy (348). In families where a child has a physical or intellectual disability, family cohesion is increased (334). Potential benefits for siblings include increased maturity, altruistic behaviour, humanitarianism and responsibility (348). Parents of children with disabilities report many of the same joys and benefits of child-rearing that parents of typically-developing children report, the so-called “common benefits” (349). Positive experiences are inversely proportional to behavioural issues and vary according to parent ethnicity (349).

Therefore, it is clear that the wellbeing of families caring for a child with a disability is a complex and multi-faceted issue. Further study is required to establish the experiences of families where there is a child with a physical disability as most of the literature focuses on intellectual disability. The mental health effects on fathers requires further study as
they are under-represented in the literature. It is also important to note that research in this area does not necessarily translate across jurisdictions due to cultural, societal and economic considerations. It is also important to consider the severity of the disability and extent of medical co-morbidities in assessing family impact. These questions must be adequately answered so that we can provide optimal support to parents and siblings of children with a disability.

1.5 Research Questions and Aims

1.5.1 Research questions

1. Is there consistency in the use of the term SNI in the medical literature? What is the definition of SNI as agreed by a multi-national panel of experts through a consensus-building Delphi process?

2. In a cohort of children with SNI, what is the extent of multi-organ dysfunction? In children with SNI, in comparison to healthy controls, is there evidence of renal or cardiac dysfunction, as measured by Cystatin C, high sensitivity Troponin T and N-terminal pro beta natriuretic peptide?

3. Do children with SNI have raised pro-inflammatory cytokines at baseline and do they display an exaggerated response to stimulation with lipopolysaccharide in vitro when compared with healthy controls?

4. Do children with SNI have reduced proportions of Neutrophils, Monocytes, Lymphocytes, or their subsets in samples of whole blood, and are these cells hyporesponsive to stimulation by lipopolysaccharide, as measured by expression of the cell surface markers CD66b, TLR4 and CD11b?

5. Do family members of children with SNI have reduced quality of life and do they perceive a significant impact on their family?

1.5.2 Aims

Aim 1: To establish a consensus-based definition for the term SNI

Children with SNI are a cohort with unique healthcare needs. The lack of a consistent definition of the term limits opportunities to capture prevalence. It may also affect communication and the usefulness of research in the area.

Deliverables:

1. A comprehensive literature review establishing whether the term SNI is used consistently in the medical literature and placing the term within the context of other commonly employed terms used to describe children with severe and complex medical needs.

2. A definition of SNI based on a consensus-building Delphi process involving a multi-national and multi-disciplinary panel of experts
Aim 2: To quantify and describe multi-organ dysfunction in a cohort of children with SNI, and to establish if these children have elevated biochemical markers of cardiac or renal dysfunction in comparison to a control population of children who do not have any chronic health conditions.

Children with SNI have complex medical issues and high healthcare usage. To date the extent of their medical complexity has not been measured. Accurate quantification of their medical complexity would allow for more effective planning of services and may assist with prognostication. Consequently, we aimed to describe and quantify multi-organ dysfunction in children with SNI.

**Deliverables:**
1. A comparison of hsTroponin T and NT-proBNP levels in serum, as markers of cardiac dysfunction, in children with SNI compared to a healthy control population of children.
2. A comparison of urea, sodium, potassium, and creatinine levels in children with SNI versus controls.
3. Cystatin C is a marker of renal function which is independent of muscle mass. We will compare Cystatin C values in children with SNI compared to controls, as a method of detecting subtle renal dysfunction in this cohort.
4. A description and quantification of the medical problems seen in each organ system in a cohort of children with SNI, attending a complex needs clinic in a tertiary children’s hospital.
5. Draft a preliminary tool for assessment of multi-organ dysfunction in children with SNI, which can be carried forward to inform a formal process by which a validated and reliable tool can be developed.

Aim 3: To describe pro-inflammatory, anti-inflammatory and hypoxia-induced cytokines, and immune cell responses, at baseline and in response to stimulation of whole blood with lipopolysaccharide, in children with SNI compared to a control group of healthy children.

Intraamniotic infection and inflammation eliciting a foetal inflammatory response and damage to the developing brain are some of the most important pathophysiological mechanisms in the development of cerebral palsy, which is likely to represent a significant proportion of those with SNI. Neonatal encephalopathy (NE) is, similarly, a significant cause of cerebral palsy (CP), accounting for 24% of cases. In both of these situations,
inflammation is known to persist for weeks, months and even years. It has been proposed that this inflammatory process along with epigenetic changes may provide a mechanism of tertiary damage. Altered inflammatory responses may also help to explain increased morbidity and mortality in this population, for example, that associated with recurrent respiratory tract infection. As a result, we aimed to detail innate immune function and inflammation in children with SNI.

**Deliverables:**

1. Measure baseline pro and anti-inflammatory cytokines in children with SNI compared to a control group of children without chronic health conditions or neurodevelopmental disabilities.
2. Examine pro-inflammatory, anti-inflammatory and hypoxia-induced cytokine responsiveness to LPS stimulation in children with SNI compared to controls.
3. Correlate LPS responsiveness with the number and type of medications, method of nutrition, number of infections, and respiratory supports seen in these children.
4. Examine differences in lymphocyte subsets, at baseline, between children with SNI and controls.
5. Evaluate the in-vitro effects of LPS on monocyte and neutrophil activation in controls compared to children with SNI.
6. Evaluate the expression of the *NLRP3, ASC* and *IL1β* genes at baseline and in response to stimulation with LPS in controls versus children with SNI.

**Aim 4: To examine if there is a perceived negative impact on quality of life or family functioning for parents or siblings who care for a child with SNI.**

Children with SNI have increased care needs which may have an impact on the family unit, including siblings. An examination of wellbeing and challenges faced by these families may allow us to intervene to improve quality of life for parents and siblings of children with SNI. We aimed to describe the wellbeing of the families of children with disabilities at the most severe end of the spectrum, i.e. those with SNI. We have correlated our findings with markers of multi-organ dysfunction to define what are the most important determinants of families’ quality of life. This may help to raise awareness of family wellbeing amongst healthcare professionals and assist with planning and allocating resources to ensure that the welfare of the family of a child with a significant disability is optimised.
Deliverables:

1. A validated and reliable questionnaire tool, the PedsQL™ Family Impact Module, will be used to measure parental quality of life and family impact in parents of children with SNI, and compared with the responses of parents of healthy controls.

2. A validated, age and developmentally appropriate questionnaire tool, the PedsQL™ Generic Core Scales 4.0, will be used to compare quality of life in siblings of children with SNI and healthy controls.

3. A focus group with teenage siblings of children with SNI will be conducted and analysed by the Interpretive Phenomenological Analysis technique, to provide a more nuanced view of the day-to-day realities of living with a brother or sister who has SNI.
Chapter 2 – Materials and Methods
2.1 Ethical approval

Ethical approval was obtained from the Research Ethics Committee of Tallaght University Hospital (Ref: 2018/09 Chairman’s Action 7; Appendix i – Ethical approval letters and amendments) and Children’s Health Ireland (CHI) at Crumlin (Ref: GEN/902/21; Appendix i – Ethical approval letters and amendments).

2.1.1 CHI at Tallaght

CHI at Tallaght is a tertiary paediatric hospital, co-located with Tallaght University Hospital. A specialist neurodisability service operates from the hospital and provides a dedicated clinic for children with complex medical needs.

2.1.2 CHI at Crumlin

CHI at Crumlin is Ireland’s largest paediatric hospital with 240 beds and 26 paediatric intensive care (PICU) beds. It provides a large proportion of the country’s tertiary and quaternary paediatric services as well as secondary services in its local catchment area. It houses the Children’s Heart Centre, the national centre for children with cardiac disorders.

2.2 Consent and Data Protection

The parents of children with SNI and controls were approached during a visit to the hospital, provided with verbal and written information about the study and asked for consent to their child’s participation (Appendix ii – Participant information leaflet; Appendix iii – Participant consent form). All parents were offered time to consider the information and were given the opportunity to ask questions. All children and young people, where appropriate, were provided with verbal and written information commensurate with their age and stage of development, following which, their assent to participation was requested.

All data was collected and processed to comply with General Data Protection Regulations (GDPR) and Health Research Regulations (HRR). Data Protection Impact assessments were performed and submitted to the Data Protection Officers of Trinity College Dublin and CHI. All patient information leaflets and consent forms were compliant with GDPR. Signed consent forms and completed questionnaires were stored in a filing cabinet in a locked office in the Trinity Centre for Health Sciences at Tallaght University Hospital. All patient data was pseudonymised, password protected and encrypted and stored on similarly-secured computer. The data key was stored on a separate desktop computer which was also password protected. Blood and serum samples were pseudonymised and those that were not analysed immediately were stored in a -80°C
freezer in a room with a coded lock in the Trinity Translational Medicine Institute (TTMI) on the campus of St. James’ Hospital, Dublin.

2.3 Definition of SNI

2.3.1 The Delphi Method and Rationale for Its Use

We chose the Delphi method as the process to reach a consensus on a definition of SNI. Since its creation the Delphi process has been adapted and used for a variety of purposes but there are a number of core elements that are common to all iterations. At its core, the process centres around panellists who are recruited for their expertise in a particular area, and operates on the assumption that group decisions are more accurate than individual ones. The process proceeds through a number of rounds, with feedback provided by a facilitator between each round. The panellists may choose to revise their answers from previous rounds, having reviewed the feedback. The group opinion tends to converge on the “correct” answer during the process. The process has several strengths which informed this choice of method: It is an accepted method for the creation of definitions in the medical literature (5, 14, 350); the ability to use an online questionnaire format was ideal for a multidisciplinary and international panel of experts; the anonymous nature of the process minimises power-dynamics which may occur for reasons such as reputation or personality and may also promote participation (351); it is cost-effective and does not require special expertise to deliver.

2.3.2 Preparatory Phase

2.3.2.1 Literature search

In preparation for the Delphi process, we carried out a review of the literature. We searched the electronic database of PubMed and the Cochrane library. The search term “Severe Neurological Impairment” was used. Searches were limited to humans. No constraints were placed on the search based on year of publication, age of subjects or type of publication. All studies with SNI included in the title were automatically included for further more detailed analysis. The titles and abstracts of the other search results were then analysed to determine relevance to our stated aims. Any articles that were of relevance were then included for more detailed analysis. This analysis included extraction of the term SNI when a definition was explicitly stated and of the term when the definition was not stated but implied by the cohort of patients included by the authors. We refer to the former as definitions of SNI, where the authors stated their interpretation of the use of the term SNI, and the latter as implied definitions of SNI, where the profile of the patients included by the authors provide insight into their understanding of the term. A search was then performed to find definitions of other terms commonly used in the literature to describe children with severe and/or complex medical needs.
2.3.2.2 Data extraction and analysis

Data were collected on whether the studies included children, adults or both. Explicitly stated definitions were extracted from each paper if present. Each definition was then broken down into phrases, which were further analysed for related concepts. These concepts were then used to generate themes, of which there were seven. The same themes were used to categorise implied definitions. Data were entered into an electronic spreadsheet and descriptive statistics were used determine frequency of the themes included in the various definitions. Themes from the literature review were used to inform the design of the survey instruments in the later stages of the Delphi process.

2.3.3 Recruitment of the Expert Panel

International experts in paediatric complex care and neurodisability were asked to assist in facilitating the Delphi process. These facilitators were well-published and prominent figures in the area of paediatric complex care, neurodisability and palliative care. This process resulted in expert facilitators in 5 countries, namely, Ireland, the United Kingdom, the United States of America, Canada and Australia.

The expert facilitators were asked to disseminate an invitation to participate in their region. A multidisciplinary panel was stipulated by the authors. The definition of “expert” was not provided but we expected that all facilitators would have a tacit knowledge of the meaning of this term. With each invitation there was a request to further disseminate the call to appropriate colleagues, employing a snowball effect in recruitment. Those who wished to participate were required to email the research team independently to express their interest; therefore, participants were anonymous.

2.3.4 Consensus

Consensus was sought on items to be included in the final definition. A priori, consensus was defined as approval (either agree or strongly agree) of 70% of participants or greater(352). Any item which reached 70% was automatically brought forward to the definition. Conversely, any item which received less than 30% approval was automatically excluded from the definition.

2.3.5 Surveys and rounds

From the outset, a limit of 3 rounds was set for the process. Each survey round was conducted online. The first round was an open round, during which, panellists were asked to list any words, phrases, themes or concepts that they associated with SNI. The first round was open for a two-week period, with a reminder email after 1 week. Free-text responses were copied verbatim, key phrases were extracted and similar responses were
grouped into themes by the author (JA) and reviewed by one of his supervisors (DMc). These themes, as well as the data that had been previously gathered from the literature review (353), were then used to inform the following rounds.

The second round sought to assess agreement with items to be included in the final definition using a 5-point Likert scale. The panellists were also invited to leave comments on each of the statements to be used in the following round as an element of feedback provided to participants. Participants were also asked to indicate wording preferences for use in the final definition. This round remained open for 3 weeks. Medians and modes of the responses were calculated using Microsoft Excel®. These statistics were then used, along with the written responses, to provide feedback to participants in round 3 of the process.

In the third round, any items which had not clearly reached consensus or had not been excluded due to non-consensus (as defined above) were brought forward. These questions were then posed again, along with feedback from the previous round. This feedback was anonymous and included selected written responses, medians and modes. Panellists were asked to vote again having reviewed this new information. The third round was open for 3 weeks. All items which had reached consensus were included in a working definition which was formulated by the research team.

2.3.6 Parental Views

Parents of children with significant disability and medical complexity were approached to give their input into the definition and two parents volunteered. A meeting was arranged between the authors and parent representatives. The process and the working definition were discussed with particular reference to wording, relevance and any areas not addressed. They felt it accurately described a cohort of children with significant disability of neurological origin, while at the same time being inclusive of those with particular underlying diagnoses and those with no specific diagnosis. Some minor wording adjustments were made.

2.3.7 Presentation at an International Meeting

The working definition was presented at the joint meeting of the American Academy of Cerebral Palsy and Developmental Medicine and the International Alliance of Academies of Childhood Disability in September 2019 (354). We used TurningPoint® audience participation software to anonymously assess agreement of attendees with the working definition. An open-floor discussion was held to inform the final definition. Wording of the definition was finalised by the research team following this meeting.
2.4 Multi-Organ Assessment in Children with SNI

2.4.1 Study population

All children less than 16 year of age who attended the neurodisability service in Children’s Health Ireland at Tallaght and who met the newly created definition of SNI were eligible for inclusion. Exclusion criteria included those who were greater than 16 years of age at the time of their outpatient clinic appointment, those who did not fit the new definition of SNI (e.g. neuromuscular disorders, not medically complex), and those who were unable to consent to participation. Blood samples were collected during routine phlebotomy.

2.4.2 Control population

The control population were recruited from the paediatric outpatient phlebotomy department in CHI at Tallaght. All healthy children under the age of 16 years were eligible for inclusion. The exclusion criteria were: children with an underlying chronic condition, including a confirmed or suspected diagnosis of a neurological disorder, signs or symptoms of acute infection, or age greater than 16 years at time of phlebotomy. Blood samples were collected during routine phlebotomy for other reasons. Controls were asked to complete the same QoL and sleep questionnaires as the families of children with SNI.

2.4.3 Clinical Data

2.4.3.1 Clinical Outcome Measures

Thorough clinical assessments were performed and healthcare records were scrutinised for basic demographic, aetiological and functional (e.g. GMFCS) information. More detailed information on involvement of each organ system was also gathered. Clinical outcome measures were recorded for each organ system as shown in Table 2.1.
Table 2.1. Clinical outcome measures recorded

<table>
<thead>
<tr>
<th>Category</th>
<th>Outcome Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis and functional information</td>
<td>• Primary Diagnosis</td>
</tr>
<tr>
<td></td>
<td>• Aetiology</td>
</tr>
<tr>
<td></td>
<td>• MRI brain (most recent)</td>
</tr>
<tr>
<td></td>
<td>• Gross Motor Function Classification System (GMFCS) (355)</td>
</tr>
<tr>
<td></td>
<td>• Manual Ability Classification System (MACS)(356)</td>
</tr>
<tr>
<td></td>
<td>• Objective assessment of cognitive ability (where available)</td>
</tr>
<tr>
<td>Neurodevelopmental</td>
<td>• History of:</td>
</tr>
<tr>
<td></td>
<td>o epilepsy and seizure type(s)</td>
</tr>
<tr>
<td></td>
<td>o dystonia</td>
</tr>
<tr>
<td></td>
<td>o dysautonomia</td>
</tr>
<tr>
<td></td>
<td>o spasticity</td>
</tr>
<tr>
<td></td>
<td>• Bulbar function</td>
</tr>
<tr>
<td></td>
<td>o Symptoms of bulbar dysfunction</td>
</tr>
<tr>
<td></td>
<td>o Documented history of aspiration</td>
</tr>
<tr>
<td></td>
<td>• Vision</td>
</tr>
<tr>
<td></td>
<td>o Cortical visual impairment</td>
</tr>
<tr>
<td></td>
<td>o Details of formal ophthalmological assessment</td>
</tr>
<tr>
<td></td>
<td>• Hearing</td>
</tr>
<tr>
<td></td>
<td>o Results of formal audiology assessment</td>
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<tr>
<td></td>
<td>• Predominant method of communication</td>
</tr>
<tr>
<td></td>
<td>• Education</td>
</tr>
<tr>
<td></td>
<td>o Special school</td>
</tr>
<tr>
<td></td>
<td>o Special Needs Assistant</td>
</tr>
<tr>
<td></td>
<td>• Number of anti-epileptic medications</td>
</tr>
<tr>
<td></td>
<td>• Number of dystonia medications</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>• History of structural or functional cardiac defects</td>
</tr>
<tr>
<td></td>
<td>• Number of cardiovascular medications</td>
</tr>
<tr>
<td>Respiratory</td>
<td>• Number of respiratory infections in past year</td>
</tr>
<tr>
<td></td>
<td>• Prophylactic antibiotics</td>
</tr>
<tr>
<td></td>
<td>• ENT</td>
</tr>
<tr>
<td></td>
<td>o History of OSA, recurrent ear infections, tympanostomy tube insertion, adenotonsillectomy</td>
</tr>
</tbody>
</table>
| Gastrointestinal | • Feeding type – oral, nasogastric, gastrostomy or jejunal  
|                  | • Weight, Height and BMI  
|                  | • History of constipation  
|                  | • History of visceral hyperalgesia, treatment and outcome  
|                  | • Number of gastrointestinal medications  
|                  | • Bloods:  
|                  |   o Liver Function Tests, Albumin |
| Endocrine        | • Vitamin D supplementation  
|                  | • Calcium supplementation  
|                  | • Pubertal assessment – normal, delayed or precocious  
|                  | • History of gynaecological issues e.g. dysmenorrhoea  
|                  | • Bloods:  
|                  |   o Vitamin D, Calcium, corrected Calcium,  
|                  |   Phosphate, Alkaline Phosphatase, |
| Orthopaedic/MSK  | • History of:  
|                  |   o Scoliosis  
|                  |   o Hip subluxation  
|                  |   o Orthopaedic surgery  
|                  |   o Botulinum Toxin administration  
|                  |   o Number of Fractures |
| Immune function  | • Number of infections in past year  
|                  | • Hospitalisations for infections in past year |
| Haematology      | • History of anaemia, thrombocytopenia or coagulopathy  
|                  | • Bloods: Full Blood Count, Ferritin |
| Dermatology      | • History of pressure sores |
| Renal/Genitourinary | • History of:  
|                   |   o Renal calculi  
|                   |   o Recurrent Urinary Tract Infection  
|                   |   o Urinary Retention  
|                   | • Prophylactic Antibiotics |
| Dental           | • Teeth grinding  
<p>|                  | • Plaque |</p>
<table>
<thead>
<tr>
<th>Genetic</th>
<th>• Results of karyotype, cytogenetics, microarray, whole exome or genome sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other</td>
<td>• Total number of medications</td>
</tr>
<tr>
<td></td>
<td>• Use of medical technology</td>
</tr>
<tr>
<td></td>
<td>• Number of hospital admissions in last year</td>
</tr>
<tr>
<td></td>
<td>• ICU admissions</td>
</tr>
</tbody>
</table>
2.4.4 Sleep Questionnaires

The Children’s Sleep Habits Questionnaire (CSHQ) is a retrospective, 35 item questionnaire which includes items relating to a number of key sleep domains in children. Items are grouped into 8 domains as follows: Bedtime Resistance, Sleep Onset Delay, Sleep Duration, Sleep Anxiety, Night Wakings, Parasomnias, Sleep-Disordered Breathing and Daytime Sleepiness(101). This questionnaire has been validated in school-aged children (101) and in toddler’s and preschool-age children (100). It has also been used widely to screen for sleep disorders in children with neurodevelopmental disorders, including cerebral palsy. (100, 104, 357, 358) The CSHQ has shown adequate test-retest reliability and internal consistency. A total score in the CSHQ of greater than 41 yields a sensitivity of 0.8 and a specificity of 0.72 for the diagnosis of a sleep disorder (101). The questionnaire was completed independently by parents. Responses were pseudonymised and scored by the PhD student (JA) using the associated scoring sheet.

2.4.5 Blood Sampling

After informed consent was obtained, whole blood samples were obtained during routine phlebotomy. Samples were taken by peripheral venous cannulation in the phlebotomy department of CHI at Tallaght. As part of routine clinical care the following were measured: Full Blood Count, Ferritin, Renal profile, Liver profile, Bone profile, vitamin D, immunoglobulins, T & B cell subsets, Troponin and Beta Natriuretic Peptide (BNP). An extra sample of whole blood (3ml) was collected in a sodium citrate anti-coagulated blood tube and transferred to TTMI for preparation and analysis. The sample arrived in TTMI within 2 hours of phlebotomy. Preparation of the sample commenced immediately and was complete within six hours of phlebotomy.

2.4.6 Whole Blood Processing

A summary of whole blood processing is shown in Figure 2.1. Whole blood was processed using 3 different methods according to the requirements for each experiment.

2.4.6.1 Processing for Experiment 1 (flow cytometry)

400uL of whole blood was divided into 100uL aliquots and placed in 4 Eppendorf tubes; 1 was stimulated with 1uL Lipopolysaccharide (LPS; E.coli 0111:B4: SIGMA Life Science, Wicklow, Ireland) 10 ng/mL, a proinflammatory agent; an equal amount of Phosphate-buffered saline (PBS) was added as a vehicle to the other 3 tubes. The samples were incubated at 37°C for 1 hour, after which time they were transferred into 4 flow tubes. A cocktail of monoclonal antibodies (mAb) to stain the cells was prepared during the incubation period. A different cocktail was used for staining Panel A (Neutrophil Panel) and Panel B (Lymphocyte panel) as shown in Table 2.2 and Table 2.3 respectively.
50uL of antibody cocktail was added to 3 of the tubes. The fourth tube remained unstained and was used for calibration of the flow cytometer later in the experiment. The samples were then incubated in the dark at room temperature for 10 minutes. Red cells were lysed with 500uL BD FACS™ 1X lysing solution (BD Biosciences, Oxford, UK) for 10 minutes in the dark. The cells were suspended in 500uL Phosphate-buffered saline with azide (PBA; PBS containing 1% bovine serum albumin and 0.02% sodium azide) to wash the cells and centrifuged (Thermo Scientific, Heraeus Fresco 17) at 1500 revolutions per minute (rpm). The supernatant was removed by pipette and discarded. The cells were then fixed with 500uL 1% paraformaldehyde (PFA) following which the cells were again washed with 500uL PBA, centrifuged at 1500rpm and the supernatant discarded. The pellet was suspended in 250uL PBA, covered in foil and stored in a refrigerator at 4°C for subsequent analysis by flow cytometry.

2.4.6.2 Processing for Experiment 2 (Cytokine analysis and Cystatin C)

Whole blood remaining after Experiments 1 and 2 was divided equally between 2 tubes; 1 was stimulated with 1uL LPS (10ng/ml) for every 100uL of peripheral blood. Both were incubated for 1 hour at 37°C, after which time they were centrifuged at 3000rpm for 10 minutes. The serum was isolated, transferred to Eppendorf tubes and frozen at -80°C(Isotemp, Fischer Scientific) for subsequent analysis of pro and anti-inflammatory cytokines and Cystatin C by enzyme linked immunosorbent assay (ELISA).

2.4.6.3 Processing for Experiment 3 (RNA extraction and analysis)

700uL whole blood was split between 2 RNA-free Eppendorf tubes; 1 was stimulated with 3.5uL LPS; 3.5uL PBS was added to the second tube. Both were incubated at 37°C for 1 hour. After incubation, 1mL of RNAlater™ was added to the samples and they were stored at -80°C for later analysis.
Figure 2.1 Flow diagram of whole blood processing. Experiment 1, flow cytometry; Experiment 2, Enzyme linked immunosorbent assay (ELISA) for cytokines and Cystatin C; Experiment 3, Ribonucleic acid extraction (RNA) and analysis; LPS, Lipopolysaccharide; rpm, revolutions per minute.
Table 2.2. Antibody panel A (neutrophil panel) for flow cytometry

<table>
<thead>
<tr>
<th>Antigen/Measurement</th>
<th>Fluorophore</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD15</td>
<td>PE-Cy7</td>
<td>2uL</td>
</tr>
<tr>
<td>TLR-4</td>
<td>APC</td>
<td>2uL</td>
</tr>
<tr>
<td>CD66b</td>
<td>Pacific Blue</td>
<td>2uL</td>
</tr>
<tr>
<td>CD16</td>
<td>FITC</td>
<td>2uL</td>
</tr>
<tr>
<td>CD14</td>
<td>PerCP</td>
<td>2uL</td>
</tr>
<tr>
<td>CD11b</td>
<td>PE</td>
<td>2uL</td>
</tr>
<tr>
<td>PBA</td>
<td></td>
<td>38uL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td></td>
<td>50uL</td>
</tr>
</tbody>
</table>

Table 2.3. Antibody panel B (lymphocyte panel) for flow cytometry

<table>
<thead>
<tr>
<th>Antigen/Measurement</th>
<th>Fluorophore</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>vδ1</td>
<td>FITC</td>
<td>2uL</td>
</tr>
<tr>
<td>vδ2</td>
<td>PE</td>
<td>2uL</td>
</tr>
<tr>
<td>CD56</td>
<td>APC-Cy7</td>
<td>2uL</td>
</tr>
<tr>
<td>CD19</td>
<td>APC</td>
<td>2uL</td>
</tr>
<tr>
<td>CD4</td>
<td>PE-Cy7</td>
<td>2uL</td>
</tr>
<tr>
<td>CD8</td>
<td>PerCP</td>
<td>2uL</td>
</tr>
<tr>
<td>CD3</td>
<td>Pacific Blue</td>
<td>1uL</td>
</tr>
<tr>
<td>PBA</td>
<td></td>
<td>37uL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td></td>
<td>50uL</td>
</tr>
</tbody>
</table>
2.4.7 Flow Cytometry

Flow cytometry is a technique which allows measurement of several single cell characteristics such as the size, granularity and intensity of fluorescence of fluorochromes bound to their surface. A laser beam strikes the cells as they move through the system. The deflection of light by the moving particles (scatter) and emission of fluorescence is measured, allowing the characterisation of cells. Forward scatter (FSC) relates to diffraction along the same axis as the laser beam and is proportional to cell size. Side scatter (SSC) measures refracted and reflected light and is proportional to granularity or cell complexity. Staining of cells with fluorescent monoclonal antibody probes allows the collection of more detailed data including quantification and characterisation of individual cells (359).

For the purposes of this study, the expression of Cluster of Differentiation (CD) 15, CD66b, CD14, CD16 and Toll-Like Receptor (TLR)-4 on the surfaces of Neutrophils and Monocytes was quantified using the following fluorochrome-labelled mAbs: CD14-PerCP, CD15-PECy7, CD16-FITC, CD66b-Pacific Blue and TLR4-APC (BioLegend®, California, USA; 2.5 μL/tube) and PE labelled CD11b (BD Biosciences, Oxford, UK; 10 μL per tube). Gamma delta 1 (γδ1) T-cells and gamma delta 2 (γδ2) T-cells were quantified and expression of CD3, CD19, CD56, CD4 and CD8 on the surfaces of lymphocytes was also measured using the following fluorochrome-labelled mAbs: γδ1-FITC, γδ2-PE, CD56-APC-Cy7, CD19-APC, CD4-PE-Cy7, CD8-PerCp. The fluorochrome-labelled mAbs used in this experiment are summarised in Table 2.2 and Table 2.3.

The prepared cells were acquired on a FACS CANTO II flow cytometer (BD Bioscience) and analysed using FlowJo Version 10 (Tree Star, Oregon, USA) software.

2.4.7.1 Quantification of cell surface antigen expression

Neutrophils were delineated based on FSC-H and CD66b positivity (+), monocytes on FSC-H and CD66b negativity (-). Monocyte subsets were based on relative CD14 and CD16 positivity: Classical (CD14+/CD16-), Intermediate (CD14+/CD16+), Non-classical (CD14dim/CD16+). Lymphocytes were selected based on SSC-A and FSC-A and subpopulations were defined as follows: B-cells (CD3-/CD19+), NK cells (CD3-/CD56+), T-cells (CD3+), CD4 (CD3+/CD4+), CD8 (CD3+/CD8+), γδ1 (CD3+/γδ1+), γδ2 (CD3+/γδ2+). At least 10,000 events were collected and relative expression of CD11b TLR4 and CD66b was expressed as mean channel fluorescence (MCF), which is the mean intensity of fluorescence emitted by all cells selected. MCF is comparable to “the number of antibodies binding to a specific cell population as they relate to the expression of receptors for ligands and/or antigen density” (360). The gating strategy is summarised for Panel A in Figure 2.2 and for Panel B in Figure 2.3. All samples were analysed by the same researcher (JA), thus reducing variability in the interpretation of results.
Figure 2.2. Gating strategy for Panel A. A) Flow cytometry dot plot showing forward scatter (FSC-A) and side scatter (SSC-A) and gated granulocytes. B) Doublets excluded by gating on single cells in FSC-H and FSC-A flow cytometry dot plot. C) Flow cytometry dot plot showing gated neutrophils and monocytes. D) Mean fluorescence intensity (MFI) of Toll-Like Receptor 4 (TLR-4) positive neutrophils. E) MFI of Cluster of Differentiation (CD)-66b positive neutrophils. F) Flow cytometry dot plot showing gated CD14 and CD16 positive subsets of monocytes. G) Flow cytometry dot plot showing gated classical, intermediate and non-classical monocytes.
Figure 2.3. Gating strategy for Panel B. A) Flow cytometry dot plot showing forward scatter (FSC-A) and side scatter (SSC-A) and gated lymphocytes. B) Doublets excluded by gating on single cells in FSC-H and FSC-A flow cytometry dot plot. C) Flow cytometry dot plot showing gated B cells. D) Flow cytometry dot plot showing gated Natural Killer (NK) cells. E) Flow cytometry dot plot showing gated T cells. F) Flow cytometry dot plot showing gated Cluster of Differentiation (CD)-4 positive T cells. G) Flow cytometry dot plot showing gated V-delta-1 T cells. H) Flow cytometry dot plot showing gated V-delta-2 T cells.
2.4.8 Multiple Enzyme-Linked Immunosorbent Assay (ELISA)

We quantified a panel of pro and anti-inflammatory cytokines using the Multiplex ELISA technique. In this technique biotinylated capture antibodies, coupled to linkers, self-assemble onto unique spots on a multiplex assay plate. Analytes in the sample then bind to the capture antibodies. Finally, detection antibodies coupled with luminescent labels bind to the analytes to complete the typical “sandwich” immunoassay. On completion of the sandwich immunoassay a voltage is applied to the assay plate, causing the captured labels to emit light, the intensity of which is measured allowing quantification of the analyte in question.

We analysed 10 individual cytokines, namely; tumour necrosis factor alpha (TNFα), interleukin 1β (IL1β), interleukin 6 (IL6), interleukin 8 (IL8), interferon gamma (IFN-γ), interleukin 18 (IL18), vascular endothelial growth factor (VEGF), erythropoietin (Epo), interleukin 1 receptor antagonist (IL1ra), and interleukin 10 (IL10). A custom-made, 96 well, 10 spot, MSD® MULTI-SPOT assay plate from Mesoscale (MSD Diagnostics, USA) was utilised.

The Uplex plate was prepared by creating the U-plex Coupled Antibody Solutions. 200ul of each individual biotinylated antibody was coupled to 300ul of a corresponding unique linker. The mixture was vortexed and incubated at room temperature for 30 minutes, after which time, 200 ul of Stop Solution was added. The solution was vortexed and incubated at room temperature for a further 30 minutes. The coating solution was prepared by adding 600ul of each U-Plex coupled antibody solution into a single tube, with pooling of the 10 antibodies listed above. Fifty microlitres of the multiplex coating solution was added to each well and the plate was sealed using an adhesive seal. Each plate was incubated for 1 hour on a plate shaker at room temperature. The plates were washed 3 times with 150ul/well of 1xMSD wash buffer solution, after which time the plate was ready for use.

To prepare the assay, 25ul of Diluent 43 was added to each well. The plate was tapped gently on all sides. 25ul of the prepared Calibrator solution was added to the calibration wells, followed by 25ul of serum. The plate was sealed and incubated at room temperature with shaking for 1 hour. After 3 washes with MSD Wash Buffer, 50ul of detection antibody solution was added to each well. The plate was sealed and incubated for 1 hour on a plate shaker at room temperature. Read Buffer was added to each well and plates were analysed on the SECTOR Imager (Meso Scale Discovery, Rockville, MD, USA; www.meso-scale.com).

2.4.9 Polymerase Chain Reaction (PCR)

Real time PCR was used to quantify the expression of 3 inflammasome-related genes i.e. NLRP-3, ASC and IL1β.
2.4.9.1 RNA extraction

Precautions

Precautions were taken throughout the process to ensure purity of the samples. All work with reagents was carried out in the hood and aseptic techniques were employed. All bench surfaces, plastic ware and pipettes were sprayed with RNAse Zap®. RNAse/DNAse-free pipette tips, microtubes and PCR tubes were used. Only RNAse-free, sterile water was used. Gloves were changed frequently and clean lab coats were worn throughout.

Cell lysis and initial purification

Whole blood samples, previously incubated at 37°C in the presence or absence of LPS (10ng/mL), were stored in RNALater™ at -80°C. The samples were allowed to thaw at room temperature. RNA was extracted using the RiboPure™ blood kit (ThermoFisher). Samples were centrifuged at 13000rpm for 1 minute and the supernatant was discarded. 800uL Lysis solution and 50uL Sodium Acetate solution was added to the cell pellet and vortexed vigorously to resuspend and lyse the cells. 400uL of Acid-Phenol:Chloroform was added to the cell lysate, vortexed for 30 seconds and incubated at room temperature for 5 minutes. The sample was then centrifuged at 13000rpm for 1 minute to separate the aqueous and organic phases. The aqueous phase containing the RNA was transferred to a new 2mL tube and the organic phase was discarded. 500uL of 100% ethanol was added to the aqueous phase and the sample was vortexed briefly.

Final purification

The sample was passed, in 700uL aliquots, through the glass filter cartridge supplied in the RiboPure™ kit. The flow-through was discarded. 700uL of Wash Solution 1 was passed through the filter. 700uL Wash Solution 2/3 was passed through the filter and this was repeated with a second 700uL aliquot of Wash Solution 2/3. The flow-through was discarded after each wash. The filter cartridge and collection tube were centrifuged at 13000rpm for 1 minute to remove residual fluid. The filter cartridge was moved to a fresh collection tube and 50uL Elution solution, preheated to 75°C, was applied. The assembly was centrifuged for 25 seconds to recover the RNA. This step was repeated with a second 50uL aliquot of Elution solution. The assembly was centrifuged for 1 minute to ensure recovery of all of the elution solution in the collection tube, after which, the filter was discarded.

DNAse digestion

DNAse I was used to remove contaminating genomic DNA from the eluted RNA. 5uL 20X DNAse buffer and 1uL DNAse I (8U/ul) was added to the eluted RNA and
incubated at 37°C for 30 minutes. 20μL DNAse Inactivation Reagent was added to the RNA, vortexed and stored at room temperature for 2 minutes. The sample was centrifuged at 13000rpm for 1 minute to pellet the DNAse Inactivation Reagent and the RNA solution was transferred to a new RNase-free tube. The NanoDrop ND-100 Spectrophotometer was used to determine RNA purity and concentration using ND-1000 ver.3.1.2 software. An absorbance curve was produced for each sample and RNA purity was calculated using the ratio of absorbance at 260nm and 280nm. Acceptable purity was considered if a ratio of \( \geq 1.6 \) RNA suspended in water was reached. Samples with a ratio less than this were discarded. The samples were stored at -20°C until cDNA synthesis was performed.

2.4.9.2 cDNA synthesis from template RNA and real-time PCR

0.1μg of RNA was reverse transcribed into a final volume of 20μl and each cDNA sample was diluted with RNAse-free water to yield a final concentration of 10ng per well. Samples were kept cool on ice throughout the process. A PCR mastermix cocktail was prepared as shown in Table 2.4. Mastermix, including 1 reaction excess.

Gene expression was evaluated using Taqman® real-time(RT) PCR. TaqMan® primer probes were used to detect expression of the following inflammasome genes: NLRP3 (NM_001079821.2), ASC(NM_013258.4) and IL-1β(NM_000576.2). GAPDH (NM_002046.3) was used as an endogenous control for data normalisation. Twenty microlitres of mastermix was added to each well of a 384-well plate. All samples were assayed in triplicate. After pipetting the samples onto the plate, it was covered and the contents were briefly centrifuged to eliminate any air bubbles. The plates were placed on ice until they were loaded onto the thermal cycler.

We employed the 7900HT Fast Real-Time PCR System (ThermoFisher Scientific), a high-throughput system which allows for detection and quantification of nucleic acid sequences. The following thermal cycling conditions were used: 2 minutes at 50°C, 10 minutes at 95°C, and, then, 24 seconds at 95°C and 1 minute at 60°C, for 40 cycles. Relative quantification (RQ) values were calculated using the \( 2^{-\Delta\Delta Ct} \) method(361), (where \( \Delta\Delta Ct \) represents the threshold cycle [Ct] of the target minus that of the internal reference [ΔCt]). Therefore, the relative amount of a gene expressed in the control is equal to 1, and the expression quantities of the stimulated counterpart are expressed relative to this (e.g. 2-fold).
Table 2.4. Mastermix cocktail

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqMan Universal PCR buffer mix</td>
<td>4uL</td>
</tr>
<tr>
<td>dNTPs</td>
<td>1.6uL</td>
</tr>
<tr>
<td>Primers</td>
<td>4uL</td>
</tr>
<tr>
<td>Enzyme</td>
<td>2uL</td>
</tr>
<tr>
<td>RNAse-free water</td>
<td>8.4uL</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>20uL</strong></td>
</tr>
</tbody>
</table>
2.5 Health Related Quality of Life (HRQoL) and Family Impact

2.5.1 Questionnaires

A number of questionnaires were used to assess health-related QoL in children with SNI and their families. For children with SNI, the Caregiver Priorities & Child Health Index of Life with Disabilities (CPCHILD) was used (362). This questionnaire is specifically designed to measure “the health status and wellbeing” of children with severe CP and has been shown to be valid and reliable(362). CPCHILD consists of 36 items grouped into 6 sections: Personal Care; Positioning, Transfer and Mobility; Communication and Social Interaction; Comfort, Emotions and Behaviour; Health; and Overall Quality of Life. A final section (Importance of Items) asks parents to rate the importance of items in the questionnaire in contributing to their child’s QoL. The questionnaires were completed independently by parents and responses were scored by the researcher using an accompanying scoring tool.

The Pediatric Quality of Life Inventory™ (PedsQL™) Version 4.0 Generic Core Scales was used to measure the wellbeing of siblings of children with SNI. PedsQL™ is a 23-item instrument which is used to measure HRQoL between the ages of 2 to 18 years. Child self-reports and parent proxy-reports are available and responses are recorded on a 5-point scale. Items are conceptually grouped into 4 subscales: Physical Functioning, Emotional Functioning, Social Functioning and School Functioning. Physical functioning can be viewed as a distinct scale while the latter 3 subscales can be combined to provide a Psychosocial Summary Scale Score(363).

The Pediatric Quality of Life Inventory™, Family Impact Module (PedsQL™ FIM) was used to measure the general wellbeing of the family of the child with SNI. The PedsQL™ FIM is a 36-item scale measuring 6 elements of parental self-reported functioning: Physical Functioning, Emotional Functioning, Social Functioning, Cognitive Functioning, Communication, Worry, and 2 elements measuring parent-reported family functioning; Daily Activities and Family Relationships(364).

Both of these PedsQL™ questionnaires are validated and reliable in children with chronic health conditions and in healthy children and have also been used to measure QoL in siblings of children with chronic health conditions (363-366).

2.5.2 Focus Group

A focus group was conducted to gain a richer understanding of the complexities of the experience of having a sibling with SNI. This focus group was conceived as a response to comments from parents and young people that questionnaires could not fully capture their lived experience. The approach used in the focus group was that of Interpretive Phenomenological Analysis (IPA). This is a qualitative research approach that
is used to explore how a person makes sense of a certain phenomenon or experience. It is particularly suited to examining topics which are complex, ambiguous and emotionally laden.

2.5.2.1 Study Population

A purposeful sample of siblings of children attending the complex needs clinic in CHI at Tallaght were recruited in June 2021. Siblings were included if they were aged between 12-18, had a sibling with SNI and lived in the same household as the child with SNI. Siblings were excluded if they had a current major psychiatric disorder, chronic illness or communication difficulty that would prevent them from participating in the study.

Participants were informed about the focus group by telephone. If they were interested in taking part a detailed parental information leaflet, consent form, age-appropriate young person’s information leaflet and assent form were posted to the family. Time was given to consider the information and there was an opportunity for participants to ask any questions they may have had regarding the study. Once fully informed consent and assent was obtained, the participants were invited to attend a focus group, held in the multi-disciplinary educational space in CHI at Tallaght. This setting is within the children’s hospital but in a non-clinical space with no medical equipment present, thus reducing any anxiety that participants may feel as a result of being in a clinical setting.

2.5.2.2 Questionnaires

The siblings were asked to complete a short questionnaire detailing some basic demographic information. Their parents were asked to complete a questionnaire outlining some demographic information about the family. If not already completed, participants and their parents were asked to complete the age-appropriate PedsQL and PedsQL FIM respectively, as detailed above.

2.5.2.3 Focus Group

The focus group was conducted by a single lead moderator, a consultant child and adolescent psychiatrist with experience and training in facilitation of such groups. An assistant moderator (JA) was also present at each focus group, with responsibility for preparation and operation of recording equipment; ensuring optimal positioning of recording equipment to maximise audibility of participant voices; note-taking; and assisting the moderator by introducing alternative perspectives, if required. The assistant moderator was a qualified physician with 7 years’ experience in paediatric medicine and 3 years’ experience researching SNI. Neither moderator were involved in the clinical care of the children with SNI or their siblings.

A semi-structured interview protocol was created following review of the literature and discussion with a multi-disciplinary team who were experienced in caring for children and young people and/or focus group methodology. All questions in the protocol were asked during the course of the focus groups.
The focus group consisted of 4 young people, both male and female. At the start of the session, the moderator recapped on the study information, outlined some basic ground rules and facilitated introductions. During the focus group, field notes were taken by the assistant moderator and the encounter was audio-recorded for later verbatim transcription. The focus group lasted approximately 110 minutes.

The focus group used open-ended questions and the moderator subsequently probed the responses to capture specific details. Departure from the agreed topics listed in the protocol was facilitated, as guided by contributions from the participants during the focus group. Throughout the interview, the moderator reflected back points raised to ensure that there was an accurate understanding of meaning. Towards the end of the sessions, the moderator summarised the main findings and asked the participants to comment, check for accuracy, provide any further insights and ask questions. The moderator remained neutral throughout the encounter and endeavoured to capture balanced views by exploring both positive and negative viewpoints and ensuring that all participants expressed their views and were heard. After the focus group the researchers held a short debriefing session to discuss the encounter, clarify any uncertainties and discuss adjustments to the protocol for potential future groups.

2.5.2.4 Data analysis

The transcribed interviews were randomly checked against the audio recordings to ensure accuracy as part of quality assurance. The transcribed interviews were analysed in a step-wise fashion using the methodology of Interpretative Phenomenological Analysis (IPA). IPA aims to explore, in detail, the sense participants make of their personal and social world and of their lived experiences through a process of interpretation, rather than by pre-existing preconceptions (369, 370). IPA is an effective methodology when exploring complex and emotionally laden topics, such as that of the siblings’ experiences (369). IPA is useful in examining this area due to the detailed attention given to enabling the participant to recount as full as possible account of their experiences.

The analysis was organised around themes which emerged from the transcript, rather than constructs predicted in advance. These themes were then considered in relation to the existing literature. As recommended by Smith (371), the transcripts were examined at least twice, once for group dynamics and patterns and then for idiographic accounts. The siblings were able to discuss their own experiences in sufficient intimacy, despite being in a group, thus the data was deemed suitable for IPA (371). At each stage of the process, the researcher continuously referred back to the transcript to ensure themes were reflective of individual experiences and emerged from the transcripts rather than derived from preconceived theory or knowledge.

IPA is a cyclical process, where the research proceeds through four iterative stages, summarised below:
Stage 1: First encounter with the text. The transcripts was read and reread to gain a general sense of the nature of the account, with notes made of potential themes (372).

Stage 2: Identifying preliminary themes. The text was reread from the beginning with emergent themes provisionally identified (372).

Stage 3: Connecting or grouping together themes. The themes were focused on in greater detail, explored to establish interrelationships with the focus on the psychological content of the phenomenon being studied and grouped together (372).

Stage 4: Tabulating themes together. The themes were coordinated to make meaningful statements that created an account of the essence and meaning of the participants' experiences, grounded in their own words (372).

2.6 Statistical Analysis

GraphPad Prism version 9.1.2 for macOS (GraphPad Software, San Diego, California, USA) and Microsoft Excel version 16.5 for Mac (Microsoft Corporation, Redmond, Washington, USA) were used for statistical analysis. Continuous data were analysed with the Shapiro-Wilk test to determine whether data were normally distributed. All normally distributed data is represented as means and standard deviations. For normally distributed data, comparison of means of 2 independent groups was performed with the student’s t-test. The one-way ANOVA test was used to compare means of 3 or more normally distributed independent groups.

In situations where Gaussian distribution could not be assumed, data is represented as medians and 95% confidence intervals. Comparison of median values of 2 independent, non-normally distributed groups were evaluated using the Mann-Whitney U test. The Kruskal-Wallis test was employed to appraise the difference in median values across 3 or more groups, while Dunn’s test was used for multiple comparisons.

Correlation between continuous, non-normally distributed data was performed using Spearman’s rank order correlation coefficient.

Logistic regression was used to test for a relationship between a binary categorical variable and an independent variable. This test assumes that the data is normally distributed, thus, if data were non-normally distributed, the data were normalised before analysing with logistic regression. Throughout the various elements of this study a p value of <0.05 was considered significant.
Chapter 3 – A Delphi Consensus-Based Definition of Severe Neurological Impairment
3.1 Introduction

SNI is a term which is commonly used in the medical literature but one for which there is no consistently applied definition. In fact, there appears to be large variation in how the term is applied, including disagreement on fundamental aspects such as whether SNI relates to disorders of the CNS, PNS or both.

The description of populations of patients is a central aspect of healthcare, both in the research and clinical setting. Accurate group descriptions allow for consistency in the performance and interpretation of research. Clinicians and other healthcare professionals decide whether research is applicable to their patients through their interpretation of the terminology used to describe a particular group. A lack of consistency in the use of terminology results in research which is less useful to the broader medical community.

A side-effect of the importance of accurate descriptors is that there is now a plethora of terms in use in all aspects of medicine. This is true of paediatric neurodisability and complex care where a wide vocabulary exists to define patient cohorts. These cohorts sometimes overlap but each can remain useful if they categorise a group of patients with unique needs. For example, there is an intersection between the groups described by the terms “neurodisability”(5) and “children with medical complexity” (CMC)(373), both of which have recognised definitions published in the literature. Some children with neurodisability will also have medical complexity, but some will not, and vice versa. Therefore, each term remains useful in its own right.

We propose the that the term SNI is a useful and relevant term which describes a group of children with unique medical needs which occur as a direct result of their underlying neurological disorder. It is an umbrella term for children with a variety of diagnoses and those with none. Their healthcare needs are likely to be significant and in excess of those of other children. A consistent definition of SNI is needed in order to capture prevalence, ensure consistency of research, optimise translation of research to clinical practice, plan services and improve healthcare outcomes for these children. Placing SNI in the context of other related definitions for children with neurological disorders or medical complexity may provide for a better conceptualisation of the importance and use of the term and, thus, improve adoption of a consensus-based definition.

3.2 Research Question

Is there consistency in the use of the term SNI in the medical literature? What is the definition of SNI as agreed by a multi-national panel of experts through a consensus-building Delphi process?
3.3 **Aim**
To establish a consensus-based definition for the term SNI.

*Deliverables:*
1. A comprehensive literature review establishing whether the term SNI is used consistently in the medical literature and placing the term within the context of other commonly employed terms used to describe children with severe and complex medical needs.
2. A definition of SNI based on a consensus-building Delphi process involving a multi-national and multi-disciplinary panel of experts

3.4 **Preparatory Phase**

3.4.1 *Literature search & included articles*
The literature review performed during the preparatory phase of the Delphi process has been published in a peer reviewed journal (353). There were 336 articles in total and 264 remained after animal studies were excluded. Thirty-four studies were automatically included for full text review as they contained SNI in the title. Following analysis of the titles and abstracts of the other articles, a further 9 studies were included, with a total of 43 papers included for full text review (61, 224, 374-414). One Japanese article was subsequently excluded as no English translation was available (389). Therefore, a total of 42 articles were analysed for their definition of SNI. Publication years of included articles ranged from 1984 to 2017. The majority (37) of the articles were published after the year 2000. Twenty-eight of the papers included children only, 5 included adults only and 9 included both.

3.4.2 *Other terms*
Seven other terms relating to children with complex or severe medical conditions are frequently used in the literature. These terms are: (1) Children with Special Healthcare Needs; (2) Children with Medical Complexity; (3) Children with Complex Chronic Conditions; (4) Medically Fragile Children; (5) Neuromuscular Disorders; (6) Children with Severe Global Developmental Delay; (7) Neurodisability. Each of these terms has a different definition (Table 3.1)

3.4.3 *Themes in the definition of SNI*
Seven themes were extracted from the various definitions of SNI in the literature. These themes were: (1) intellectual disability; (2) mobility; (3) communication ability; (4) dependence on others for decision-making; (5) chronicity; (6) involvement of the central
nervous system or peripheral nervous system; and (7) care needs in excess of those of typically developing children.

3.4.4 Definitions of SNI

3.4.4.1 Explicitly-stated definitions

Twenty-three papers included explicitly stated definitions of SNI (Table 3.2). We examined each of these definitions and assessed whether a statement on the seven previously stated themes was included (Table 3.3).

Motor impairment was most consistently included in stated definitions of SNI, with impairment of mobility a criterion in just over 80%. Intellectual disability was the next most consistent inclusion criterion, appearing in 70% of definitions (excluded in 20%). Impairment of communication is included in 30% of definitions and increased care needs in 13%.

Four definitions of SNI included a statement of chronicity, 3 definitions referred to acute neurological impairment and there was no distinction in the others. Disorders of the CNS were specified in the definition of 4 of the studies, while none explicitly referred to SNI as a disorder of the PNS. Reliance on others for decision-making was included in the definition of SNI in 2 cases.

3.4.4.2 Implied definitions of SNI

We analysed each paper where the definition was not explicitly stated to understand implied definitions and explored whether patients relating to each definitional theme were included, excluded, or had both categories included in them. In many cases, there was not enough information to decipher into which category the participants fell (Table 3.4).
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
</table>
| Children with Special Healthcare Needs (CSHCN) (1)                  | A chronic physical, developmental, behavioral, or emotional condition  
Require health and related services of a type or amount beyond that required by children generally. |
| Children with Medical Complexity (CMC) (373)                        | Coexistence of multiple family-identified service needs, chronic conditions, functional limitations, and extraordinarily high health care use  |
| Children with Complex Chronic Conditions (CCC) (415)                | Medical condition... expected to last at least 12 months (unless death intervenes)  
Involves either several different organ systems, or 1 organ system severely enough to require specialty pediatric care |
| Medically Fragile Children (MFC) (416)                              | Dependent on technology for survival                                                                                                                                                                |
| Neuromuscular Disorders (NMD) (417)                                 | Disorders that involve injury or dysfunction of peripheral nerves or muscle                                                                                                                            |
| Children with severe global developmental delay (SGDD) (418)        | Significant intellectual disability and severe motor impairment; Extremely limited functional movement; Dependent upon others for all activities of daily living |
| Neurodisability (5)                                                 | “A group of congenital or acquired long-term conditions which are attributed to impairment of the brain and/or neuromuscular system and create functional limitations. A specific diagnosis may not be identified. Conditions may vary over time, occur alone or in combination, and include a broad range of severity and complexity. The impact may include difficulties with movement, cognition, hearing and vision, communication, emotion, and behaviour” |
## Table 3.2. Definitions of SNI.

<table>
<thead>
<tr>
<th>Source</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakke et al (374)</td>
<td>neurological disorders but no significant mental deficiencies</td>
</tr>
<tr>
<td>Buratti et al (377)</td>
<td>severe psychomotor and mental retardation</td>
</tr>
<tr>
<td>Danielson &amp; Emmens (380)</td>
<td>non-ambulatory\non-ambulatory severe psychomotor and mental impairment</td>
</tr>
<tr>
<td>Elliott (381)</td>
<td>Profoundly, irreversibly neurologically damaged\Will never be able to speak, to walk, to sit up, or to feed themselves\Intellectual abilities extremely limited</td>
</tr>
<tr>
<td>Hauer (384)</td>
<td>Nonverbal\Severe impairment of the central nervous system</td>
</tr>
<tr>
<td>Hauer &amp; Solodiuk (224)</td>
<td>Severe global impairment of the CNS</td>
</tr>
<tr>
<td>Khoshoo et al (388)</td>
<td>Profound developmental delay\Neuromuscular impairment\Nonverbal and wheelchair-bound</td>
</tr>
<tr>
<td>Lind et al (390)</td>
<td>Glasgow Outcome Scale (GOS) III and IV</td>
</tr>
<tr>
<td>Mahant et al (392)</td>
<td>Gross Motor Functional Classification System (GMFCS) III-V</td>
</tr>
<tr>
<td>McCrea et al (395)</td>
<td>Profound developmental impairment\Requiring constant care\Cannot mobilise\Very limited possibilities for communication</td>
</tr>
<tr>
<td>Mergler et al (397, 398)</td>
<td>Moderate or severe intellectual disability (intelligence quotient &lt;55)\Gross motor functioning classification system level IV or V</td>
</tr>
<tr>
<td>Orel et al (419)</td>
<td>Gross Motor Function Classification system (GMFCS) grade V</td>
</tr>
<tr>
<td>Pinnington et al (402, 403)</td>
<td>Quadrupedal distribution of athetosis and/or spasticity, Associated impairments in visual perception and learning</td>
</tr>
<tr>
<td>Roka et al (404), Sun et al (409)</td>
<td>mental development index or physical development index &lt;70</td>
</tr>
<tr>
<td>Siden et al (406)</td>
<td>Cognitive impairments\Communication impairments</td>
</tr>
<tr>
<td>Study</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
</tr>
</tbody>
</table>
| Smith et al<sup>(407)</sup> | Serious neurological disease  
Continuing neurological symptoms and signs |
| van Gestel et al<sup>(411)</sup> | Significant limitations both in intellectual functioning and in adaptive behaviour as expressed in conceptual, social, and practical adaptive skills, originating before the age of 18 years  
Classified as severely impaired when they had profound developmental delay, required around-the-clock care and support, were completely dependent on a wheelchair and had no, or very limited, possibilities for communication |
| Winfield et al<sup>(412)</sup> | Significant intellectual disability  
Severe motor impairment  
Extremely limited functional movement  
Dependent upon others for all activities of daily living |
| Yamazaki et al<sup>(413, 414)</sup> | Mental retardation and/or  
Motor disabilities |
Table 3.3. Frequency of themes in published definitions of SNI.

<table>
<thead>
<tr>
<th>Theme</th>
<th>Included n</th>
<th>Excluded n</th>
<th>Not mentioned n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intellectual disability</td>
<td>16</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Limitations in mobility</td>
<td>19</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Communication difficulties</td>
<td>7</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Increased care needs</td>
<td>3</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>CNS vs PNS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dependent n</td>
<td>4</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Independent n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dependence on others for decision-making</td>
<td>2</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Chronicity</td>
<td>4</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Chronic n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute n</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.4. Frequency of themes in implied definitions of SNI

<table>
<thead>
<tr>
<th>Theme</th>
<th>Included n</th>
<th>Excluded n</th>
<th>Both n</th>
<th>Not enough information n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intellectual disability</td>
<td>20</td>
<td>7</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Limitations in mobility</td>
<td>27</td>
<td>2</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Communication difficulties</td>
<td>15</td>
<td>6</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>Increased care needs</td>
<td>20</td>
<td>5</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>CNS vs PNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS n</td>
<td>33</td>
<td>0</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>PNS n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not enough information n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decision-making</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dependent n</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Independent n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not enough information n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic n</td>
<td>33</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Acute n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thirty-three papers included only chronic conditions, 6 included only acute presentations of neurological disease, i.e. acute brain injury, while 1 paper included both chronic and acute conditions. In the remaining papers, there was not enough information to differentiate acute from chronic disorders. Thirty-three of the articles referred to patients with CNS disorders only, while 5 included both patients with disorders of the CNS and PNS. None of the studies included only those with disorders of the PNS although 4 of them did not have enough information to make a determination on this aspect of the definition. Eight papers involved participants who rely on others for decision-making, 5 involved those capable of making their own decisions and 1 included both categories of participants.

Within the implied definitions of SNI, impaired mobility again appeared central to the use of the term, with only 19% of papers not including it in patient selection. Intellectual disability was considered essential in 48% of articles, impaired communication in 36% and increased care needs in 48%. CNS disorders were included more frequently than PNS disorders and chronicity more frequently than acute conditions, especially in the paediatric population. Again, the reliance on others for decision-making did not appear to be a vital component in the selection of patients with SNI.

3.5 The Delphi Process

Following the literature review, the Delphi process was identified as the most appropriate method of reaching consensus on a definition of SNI. The process proceeded through three rounds, as described in Chapter 2. The results of the process are described below and summarised in Figure 3.1 and Table 3.5.

3.5.1 Round one of the Delphi process

Thirty-eight healthcare professionals responded to the initial invitation to participate in the process. Thirty-four panellists completed the first round within the specified time period. Twenty-two panellists were working in Ireland, six in the USA, four in Australia, one in Canada and one in the UK. Fourteen physicians, 14 allied health professionals (Occupational Therapy (OT); Physiotherapy; Speech and Language Therapy (SLT); Dietetics) and 6 nurses took part.

Nineteen themes were identified from the responses in round 1 of the Delphi process and the literature review, namely: motor impairment; aetiology; cognitive impairment; quality of life; central nervous system (CNS) versus peripheral nervous system (PNS); communication; medical complexity; sensory impairments; increased care needs; dependence on medical technology; multi-organ dysfunction; progressive or static; emotional impairment; chronicity; a quantification of severity; family-reported issues; life limitation; social functioning; and dependence for decision-making.
3.5.2 Round two of the Delphi process

In the second round, 30 panellists participated. However, 1 of these panellists, having commenced the process, did not provide responses to the questions; therefore, 29 participants completed round 2. This included 12 physicians, 5 nurses and 13 allied health professionals (2 SLT, 4 physiotherapists, 5 OT, 1 dietician and 1 clinical psychologist).

More than 70% of participants agreed that the following themes were essential to the definition of SNI: motor impairment (82.8%); cognitive impairment (79.3%); medical complexity (75.9%); increased care needs (89.7%); and that it may include disorders that are progressive or static (89.7%). The following themes were automatically excluded as they reached less than 30% agreement: Emotional impairment (13.8%); dependence on medical technology (24.1%); multiorgan dysfunction (20.7%); family-reported issues (27.6%). It is important to note that dependence on medical technology, multiorgan dysfunction and family-reported issues often form part of the term “medical complexity”, as does increased care needs, but participants were asked whether these terms should be included, independently of medical complexity, to emphasise their importance. Nine items were brought forward to round 3 for participants to vote upon again, following feedback, as previously described. The items brought forward were: aetiology (48.3%); CNS versus PNS (44.8% voted CNS alone); quality of life (44.8%); life-limitation (34.5%); communication (51.7%); social functioning (55.2%); sensory impairments (41.4%); quantification of severity (62.1%); and dependence for decision-making (34.5%). One item, chronicity, was excluded after round 2 as the clear agreement that the disorder could be progressive or static rendered this theme irrelevant.

3.5.3 Round three of the Delphi process

Thirty-one panellists participated in round 3 of the Delphi process. One participant answered the first question only; therefore, for the remaining questions, the denominator was 30. The panel included 13 physicians, 6 nurses and 12 allied health professionals (2 SLT, 4 physiotherapists, 4 OT, 1 dietician and 1 clinical psychologist).

Following round 3, one further item was included in the definition, namely that SNI referred to disorders of the CNS (73.3%). All other items (listed below) saw a decline in agreement; thus, did not reach the 70% cut-off for consensus and were excluded from the final definition. The items excluded after this round were: aetiology (33.3%); quality of life (10%); life-limitation (26.7%); communication (40%); social functioning (36.7%); sensory impairments (26.7%); quantification of severity (46.7%); and dependence for decision-making (26.7%).
Preparatory phase including literature review

Delphi process established as most appropriate method to reach consensus

Round 1 of Delphi process
Open responses
\( n = 34 \)

Recruitment of expert panellists
Purposive sampling and snowballing
International and multi-disciplinary

Thematic analysis and preparation for round 2

Round 2 of Delphi process
Likert scales
\( n = 29 \)

Data processing and analysis
Consensus – 5 items
Excluded – 4 items
Removed – 1 item

Round 3 of Delphi process
Feedback provided
Likert scales
\( n = 31 \)

Data processing and analysis
Consensus – 1 item
Excluded – 8 items

Working definition created with input from parent representatives

Presentation at international conference with open-floor discussion

Finalised definition created

Figure 3.1. Stages of the Delphi process
Table 3.5. Summary of results from rounds 1-3 of the Delphi process

<table>
<thead>
<tr>
<th>Number of participants</th>
<th>Round 1 n</th>
<th>Round 2 n (%)</th>
<th>Round 3 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Profession</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician</td>
<td>14</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Nurse</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Allied health professional (total)</td>
<td>14</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Physiotherapist</td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Occupational Therapist</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Speech and Language Therapist/Speech Pathologist</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dietician</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clinical Psychologist</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Country</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>22</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>USA</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Australia</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>UK</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Canada</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Themes</th>
<th>Round 1 n</th>
<th>Round 2 n (%)</th>
<th>Round 3 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor impairment</td>
<td>24 (82.8)</td>
<td>Included after R2</td>
<td></td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>23 (79.3)</td>
<td>Included after R2</td>
<td></td>
</tr>
<tr>
<td>Aetiology</td>
<td>14 (48.3)</td>
<td>10 (32.3)</td>
<td></td>
</tr>
<tr>
<td>Quality of life</td>
<td>13 (44.8)</td>
<td>3 (10)</td>
<td></td>
</tr>
<tr>
<td>Life limitation</td>
<td>10 (34.5)</td>
<td>8 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Social functioning</td>
<td>16 (55.2)</td>
<td>11 (36.7)</td>
<td></td>
</tr>
<tr>
<td>CNS vs PNS vs both CNS alone</td>
<td>13 (44.8)</td>
<td>22 (73.3)</td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>PNS alone</td>
<td>Both</td>
<td>Do not specify</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-----------</td>
<td>------</td>
<td>---------------</td>
</tr>
<tr>
<td>Communication</td>
<td>15 (51.7)</td>
<td>12 (41.4)</td>
<td></td>
</tr>
<tr>
<td>Medical complexity</td>
<td>22 (75.9)</td>
<td>Included after R2</td>
<td></td>
</tr>
<tr>
<td>Multi-organ dysfunction</td>
<td>6 (20.7)</td>
<td>Excluded after R2</td>
<td></td>
</tr>
<tr>
<td>Dependence on medical technology</td>
<td>7 (24.1)</td>
<td>Excluded after R2</td>
<td></td>
</tr>
<tr>
<td>Family-reported issues</td>
<td>8 (27.6)</td>
<td>Excluded after R2</td>
<td></td>
</tr>
<tr>
<td>Increased care needs</td>
<td>26 (89.7)</td>
<td>Included after R2</td>
<td></td>
</tr>
<tr>
<td>Sensory Impairments</td>
<td>12 (41.4)</td>
<td>8 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Dependence for decision making</td>
<td>10 (34.5)</td>
<td>8 (26.7)</td>
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</tr>
<tr>
<td>Emotional Impairment</td>
<td>4 (13.8)</td>
<td>Excluded after R2</td>
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<tr>
<td>Quantification of severity</td>
<td>18 (62)</td>
<td>14 (46.7)</td>
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<tr>
<td>Progressive vs static vs both</td>
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<td>Progressive alone</td>
<td>1 (3.4)</td>
<td>2 (6.9)</td>
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</tr>
<tr>
<td>Static alone</td>
<td></td>
<td></td>
<td>26 (89.7)</td>
</tr>
<tr>
<td>Both</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>19 (67.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>1 (3.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>8 (28.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do not specify</td>
<td>1 (3.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CNS = Central Nervous System; PNS = Peripheral Nervous System; R2 = Round 2; Items in **bold** reached the consensus threshold and were included in the final definition.
3.6 Finalising the definition

After the third round was complete, a working definition of SNI was created. The working definition was as follows (Figure 3.2):

“Severe Neurological Impairment is a descriptive term for a group of children with disorders of the central nervous system, resulting in motor impairment, cognitive impairment and complex medical needs, who require much assistance with their activities of daily living. The impairment is permanent but can be progressive or static.”

Figure 3.2. Working definition of Severe Neurological Impairment

The definition was presented at an international, multi-disciplinary conference and audience response software was used to gauge agreement with our working definition. Fourteen attendees chose to download and interact via the software. All participants agreed, in general, with the working definition. An open floor discussion followed and comments from the audience were invited on the working definition. After the conference, and using the feedback received, 2 items from the working definition were slightly altered for the final version. “A group of children” was changed to “which arise in childhood” to reflect that children with SNI can progress to become adults with SNI; “complex medical needs” became “medical complexity”. This latter change was made to better align the term medical complexity with the definition of CMC as proposed by Cohen et al (i.e. “substantial family-identified health care service needs”, “1 or more chronic clinical condition(s)”, functional limitations and high health care use (373), in an attempt to avoid ambiguity. Thus, the final definition is as follows (Figure 3.3):

“Severe Neurological Impairment describes a group of disorders of the central nervous system which arise in childhood, resulting in motor impairment, cognitive impairment and medical complexity, where much assistance is required with activities of daily living. The impairment is permanent but can be progressive or static.”

Figure 3.3. Finalised definition of Severe Neurological Impairment

3.7 Discussion

3.7.1 Literature Review

The review of the literature demonstrated inconsistency in the use of the term Severe Neurological Impairment. Several definitions exist to describe children with severe and complex medical conditions. These definitions are often broad and include children with a wide variety of difficulties. For example, CMC may describe children with conditions as diverse as complex congenital cardiac disease, chronic renal impairment or cerebral palsy. Children with SNI may be included within these defined groups. However, given
their unique health and social care needs in the context of neurological impairment, an agreed definition distinguishing this group from others would be helpful as a starting point to assess need and plan services.

Many of the terms we found to describe children with complex medical conditions have been widely agreed and appear to be consistently used. One exception to this is the term “Neuromuscular Disorders” (NMD). The definition we have used in the literature review (Table 3.1) uses NMD to describe disorders of the PNS and muscle. However, an alternative use of the term employs International Classification of Diseases, 9th Revision (ICD-9) codes to create a list of disorders that fit into this category(415). The latter use of NMD includes disorders of the CNS as well as the PNS, such as epilepsy, “mental retardation”, and brain and spinal cord malformations. These differing views on what constitutes neuromuscular disorders may explain the variable inclusion of central and peripheral pathology in exploring the concept of severe neurological impairment. The exclusion of exclusively PNS disorders within the rubric of SNI in the literature is of interest and may reflect the relative importance given to intellectual disability and primary communication difficulties within the SNI concept.

From the literature, in definitions of SNI, the most important considerations appeared to be issues with mobility, intellectual disability, communication difficulties and increased care needs. Distinction between acute versus chronic, CNS versus PNS disorders, and dependence on others for decision-making appeared to be less important, as they were not clearly addressed in most cases. However, there remained considerable variation between authors as to which criteria are essential for the classification of SNI.

We have shown considerable inconsistency in the use of the term SNI. However, the creation of an internationally-accepted, consensus-based definition presented many difficulties. Each component of the term can be challenging to define on its own, particularly when the complexities of cultural differences in interpretation of concepts are taken into account. We have already discussed one of these components; namely the use of the word “neurological” and variation in whether this comprises CNS, PNS or both. The term “severe” can, in itself, be difficult to quantify; when does an impairment cease being moderate and become severe? Similarly, “impairment” may be open to differences in interpretation depending on one’s cultural background. However, this final difficulty may have already been resolved in the World Health Organisation’s International Classification of Functioning, Disability and Health (ICF), in which they define the term impairment as “problems in body function or structure such as a significant deviation or loss”(420). Additionally, it was important to consider whether Severe Neurological Impairment included chronic conditions alone or whether acute neurological impairment can also be included in this category. From our review of the literature, it was clear that this issue remained to be resolved; although most of the definitions related to chronic conditions,
some used the term SNI to refer to acute conditions only, and others included a combination of both. In agreeing a definition we aimed to resolve this issue, as well as addressing the inclusion of progressive disorders.

### 3.7.2 A Delphi Consensus-Based Definition

A consensus-based definition of SNI has been created through this Delphi process. As discussed above, SNI is a term which is commonly used in the medical literature but is not used consistently (353). Lack of an agreed definition may result in variance in reporting of prevalence and outcome measures. This is an issue which has previously been demonstrated in the literature with other terminology such as “disability”. For example, in 2004, Bajekal et al. published a review of disability estimates and definitions in the UK (421). They found that estimates of adults with disabilities varied from 8.6 million to 11 million, with much of this variation attributed to how disability is defined.

The development of a consensus-based definition of SNI should improve the precision of reporting of research and, consequently, may improve outcomes in health, quality of life, as well as decision making at the end of life (EOL) for those with SNI.

Strengths of this definition of SNI include that it was developed in a systematic manner, including literature review, expert opinion and lived-experience. An open round was used to reduce bias. Criteria for consensus were defined a priori in line with best practice; consensus was set at 70% agreement, a generally accepted benchmark (352). The feedback between the second and third round was both numerical and narrative. Narrative responses were anonymised and represented different points of view to avoid the introduction of bias. The provision of both forms of feedback provided the opportunity to consider all points of view, including some more subtle differences in opinion. Hallowell (422), citing Best (423), describes how this technique improves accuracy in the Delphi technique.

Another significant strength of this definition is the focus on diseases and injury of the central nervous system, a group of disorders with risk for multiple comorbid problems not seen with peripheral disorders. Other terminology combines these 2 distinct groups (373, 424, 425). Such a definition can also benefit children with impairment of the CNS yet without a specific diagnosis. Going forward, including subcategories of intellectual and motor disability will be an essential part of health outcomes research.

A multi-disciplinary panel of experts was included to incorporate a diverse range of expertise and to ensure that the definition was useful across the disciplines. The physician and allied health professional groups had similar numbers, while it would initially appear that nurses had fewer representatives. However, both the physician and allied health professional groups are relatively heterogeneous i.e. physicians include experts in paediatric palliative care, neurodisability, community paediatrics etc. On the other hand,
the nursing group is quite homogenous and was comprised of nurses with an interest in caring for children with neurodisability and/or medical complexity.

The views of international experts were sought and participants from 5 countries signed up to participate. However, we acknowledge that there are limitations to the panel that was recruited. All participants were from English-speaking countries and there were no participants from middle or low-income countries. Therefore, the resulting definition may not be considered truly international. It was also noted that panellist participation was skewed towards one country which may also be considered a limitation of the study. Some differences in terminology were noted in this process, including the use of cognitive impairment rather than intellectual disability in the definition. Although efforts were made to ensure that the panel was multi-disciplinary, we also acknowledge that there are professionals involved in the care of children with SNI who were not included in our panel. For example, professionals in the educational sector may have provided useful and alternative insights into the definition of SNI. These issues would be important to address in any future work on updating the definition of SNI.

Although there were a relatively small number of participants, this is considered appropriate in Delphi methodology and produces reliable results(426). Thus, a large panel size is not considered vital to render the conclusions of the Delphi process valid.

Perhaps, a more important consideration in Delphi reliability is participant attrition. Panellist dropout is acknowledged to be a major problem in the Delphi process and may cause response bias(427). The dropout rate from round 1 to round 3 was only 9%, so, retention of our expert panellists throughout the process was excellent and could be considered a further strength of the study.

Although many items did not reach the level of consensus for inclusion in the definition of SNI, they are still important considerations in the clinical care of these children. It is important to emphasise that this definition is a descriptive term for a group of children with similar issues and is not intended as a diagnosis. The endorsement of the definition of SNI by professional organisations in the future will be important to ensure that the definition is adopted widely. Definitions should not be static and it is probable that this definition will need to be adapted in the future. In particular, future directions should involve input from developing countries and from experts in other professional fields such as education.
Chapter 4 – Cytokine responses in Severe Neurological Impairment
4.1 Introduction

Children with Severe Neurological Impairment (SNI) have disorders of the central nervous system (CNS) which result in significant motor and cognitive impairment as well as medical complexity (428). They are a heterogeneous group of children which includes those with a definitive (sometimes rare) diagnosis and those with none. They are unified by the severity of their functional limitations and complex medical needs which occur as a direct result of CNS dysfunction. The CNS abnormality may be congenital e.g., a neuronal migration disorder, or it may be acquired e.g., neonatal encephalopathy (NE).

Children with neurological and neurodevelopmental disorders have altered inflammatory responses (127, 429-433). Infants with NE have alterations in serial cytokine measurements in the first week of life which correlated with mortality (431). These abnormalities appear to persist into school-age and elevated Tumour Necrosis Factor (TNF)-β was associated with poorer gross motor function (127). Children with one of the most well-known neurodevelopmental disorders, Down syndrome, have altered cytokine levels which may be related to their increased susceptibility to sepsis (433). Recent research has also pointed to altered inflammatory responses in school-age children with cerebral palsy (CP) (429).

Cytokines are proteins which have a variety of functions within the immune system. The cytokines secreted in response to an insult regulate the nature and strength of the immune response. Many cytokines have traditionally been considered either pro or anti-inflammatory, though the reality is much more complex, and several cytokines may have both pro and anti-inflammatory effects depending on its source, target, and timing in the immune response (434). Cytokines are, therefore, integral to the strength and quality of the immune response and a delicate balance is required to ensure health.

Abnormalities in the cytokine response to an insult may have significant impact on health-related outcomes such as morbidity related to recurrent infections or sepsis-related mortality. In CP, those with the most severe disability have been shown to have a 50% mortality by the age of 15 years (260). Death was attributed to respiratory problems in 56.8% of which 82% had pneumonia and a further 16% died from non-respiratory infections (260). Therefore, almost half of deaths in those with CP were attributable to infection. This does not consider cases where infection may have played a role in precipitating the primary cause of death e.g., in those with “> 1 sufficient cause of death” recorded, half had complicated pneumonia. The reasons for this are multi-faceted, but an altered inflammatory state with altered cytokine responses may be a potential contributor.

Neurodevelopmental outcome may also be influenced by alteration of the inflammatory response. Activation of the immune system in early life is associated with several neurodevelopmental disorders including autism, schizophrenia, and CP (435). Inflammation, through epigenetic modifications, is postulated to play a role in tertiary
neurological injury. Improving our knowledge of inflammatory responses in children with neurodevelopmental disorders could lead to discovery of potential therapeutic targets which may improve neurological outcome.

We sought to broaden our understanding of cytokine response to a lipopolysaccharide (LPS)-induced immune activation in children with SNI. We then evaluated for correlation between these cytokine responses and other surrogate markers of health such as number of regular medications, infections and hospital admissions in the past year, and sleep scores.

4.2 Hypothesis

Children with SNI have higher levels of pro-inflammatory cytokines in serum at baseline and are hyperresponsive to stimulation with lipopolysaccharide when compared to a control population of healthy children.

4.3 Aims

To describe pro-inflammatory, anti-inflammatory and hypoxia-induced cytokines in serum, at baseline, and in response to stimulation of whole blood with lipopolysaccharide, in children with SNI compared to a control group of healthy children.

Deliverables:

1. Measure baseline pro and anti-inflammatory cytokines in children with SNI compared to a control group of children without chronic health conditions or neurodevelopmental disabilities.

2. Examine cytokine responsiveness to LPS stimulation in children with SNI compared to controls.

3. Correlate LPS responsiveness with the number and type of medications, method of nutrition, number of infections, and respiratory supports seen in these children?

4.4 Results

4.4.1 Demographics and Clinical Characteristics

All participants were recruited from Children’s Health Ireland (CHI) at Tallaght. Participants in the control group (n=14) were healthy children (age range 1.25-15.25 years), who were undergoing phlebotomy. None had an active infection, chronic disease or neurodevelopmental disorder. There were no significant differences in age or sex between the control and SNI group (Table 4.1).

Children with SNI (n=15), (age range 1.84-16.5 years) who were attending the Complex Needs Clinic in CHI at Tallaght were recruited to the SNI group. This included
children with a variety of neurodevelopmental disorders, including CP (n=10) of varying aetiology, Wolf-Hirschhorn Syndrome (n=2), Rett Syndrome (n=2) and Calcium/Calmodulin Dependent Serine Protein Kinase (CASK) mutation (n=1) (Table 4.1). All children in the SNI group had motor impairment, cognitive impairment, and medical complexity as a result of a permanent disorder of the central nervous system (428). All children in the SNI group were classed as having Gross Motor Function Classification System (GMFCS) IV-V. They had medical co-morbidities including, but not limited to, epilepsy (n=11), visual impairment (n=8), hearing impairment (n=3), recurrent respiratory tract infections requiring prophylaxis (n=4), a requirement for respiratory supportive technology (n=5) and/or enteral feeding tube (n=9) (Table 4.1). Children in the SNI group were prescribed a mean of 6.64 ± 3.57 regular medications.

4.4.2 Cytokine Results

Several pro-inflammatory and anti-inflammatory cytokines were analysed as were a number of markers of hypoxia as follows: Erythropoietin (EPO), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), Interferon Gamma (INF-γ), Interleukin (IL)-10, IL-18, IL-1 Receptor Antagonist (RA), IL-1α, IL-1β, IL-2, IL-6, IL-8, Tumour Necrosis Factor (TNF)-α, TNF-β and Vascular Endothelial Growth Factor (VEGF).

There were no significant differences in baseline values between the control and SNI cohorts (Table 4.3 & Figure 4.1). No significant increase in cytokine level was seen in either the control or SNI cohorts for EPO (p>0.99; p=0.71), INF-γ (p=0.84; p>0.99), IL-18 (p>0.99; p>0.99) or TNF-β (p=0.58; p>0.99) (Table 4.3 & Figure 4.1).

Significant increases were seen after addition of LPS in both the control and SNI groups in the following cytokines, indicating an appropriate response to LPS stimulation: IL-10 (p=0.02; p=0.0005), IL-1ra (p<0.0001; p=0.0007), IL-1β (p=0.0008; p=0.0023), IL-6 (p=0.0002; p=0.02), IL-8 (p=0.001; p<0.0001), TNF-α (p=0.001; p<0.0001) and VEGF (p=0.001; p=0.00004) (Table 4.3 & Figure 4.1). The level of GM-CSF increased in the control group (p=0.04) but not in the group of children with SNI (p=0.07), indicating an element of hyporesponsiveness to LPS in the latter.

The SNI cohort had a relatively larger increase in EPO in response to LPS than the comparison group with a median increase of 36.77% (95% CI 22.16-62.42) versus 16.53% (95% CI 7.44-28.86) respectively (p=0.0068). IL-6 in the SNI cohort was relatively hyporesponsive to LPS with a median increase of 443.1% (95% CI 216.9-728.7) versus 1106% (95% CI 541.9-2320) respectively (p=0.012). There were no significant differences in the relative change of cytokine level before or after LPS stimulation for any of the other markers studied (Table 4.4 & Figure 4.2).
Table 4.1. Age and sex characteristics of Control and SNI group. SNI, Severe Neurological Impairment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>SNI group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males n (%)</td>
<td>7 (50)</td>
<td>7 (46.67)</td>
<td>N/A</td>
</tr>
<tr>
<td>Age mean (SD)</td>
<td>8.11 (4.45)</td>
<td>11.41 (4.78)</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Table 4.2. Clinical characteristics of children in the SNI group. SNI, Severe Neurological Impairment; CP, Cerebral Palsy; CASK, Calcium/Calmodulin Dependent Serine Protein Kinase; GMFCS, Gross Motor Function Classification System; VNS, Vagal Nerve Stimulator; RTI, Respiratory Tract Infection; PEG, Percutaneous Endoscopic Gastrostomy; PEJ, Percutaneous Endoscopic Jejunostomy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%) unless otherwise stated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>10 (66.67)</td>
</tr>
<tr>
<td>-Dyskinetic</td>
<td>4</td>
</tr>
<tr>
<td>-Spastic</td>
<td>4</td>
</tr>
<tr>
<td>-Mixed</td>
<td>2</td>
</tr>
<tr>
<td>Wolf Hirschhorn Syndrome</td>
<td>2 (13.33)</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>2 (13.33)</td>
</tr>
<tr>
<td>CASK mutation</td>
<td>1 (6.67)</td>
</tr>
<tr>
<td><strong>Aetiology of CP</strong></td>
<td></td>
</tr>
<tr>
<td>Neonatal Encephalopathy</td>
<td>3</td>
</tr>
<tr>
<td>Congenital brain malformation</td>
<td>2</td>
</tr>
<tr>
<td>Infection</td>
<td>2</td>
</tr>
<tr>
<td>Prematurity</td>
<td>2</td>
</tr>
<tr>
<td>Genetic</td>
<td>1</td>
</tr>
<tr>
<td><strong>GMFCS (%)</strong></td>
<td></td>
</tr>
<tr>
<td>I-III</td>
<td>0 (0)</td>
</tr>
<tr>
<td>IV-V</td>
<td>15 (100)</td>
</tr>
<tr>
<td><strong>Intellectual disability</strong></td>
<td>15 (100)</td>
</tr>
<tr>
<td><strong>Epilepsy</strong></td>
<td></td>
</tr>
<tr>
<td>Requiring 0-2 anti-epileptic medications</td>
<td>5</td>
</tr>
<tr>
<td>Requiring &gt;2 anti-epileptic medications/VNS</td>
<td>6</td>
</tr>
<tr>
<td><strong>Visual Impairment</strong></td>
<td>8 (53.33)</td>
</tr>
<tr>
<td><strong>Hearing Impairment</strong></td>
<td>3 (20)</td>
</tr>
<tr>
<td><strong>Recurrent RTI requiring prophylaxis</strong></td>
<td>4 (26.67)</td>
</tr>
<tr>
<td><strong>Respiratory supportive technology</strong></td>
<td>5 (33.33)</td>
</tr>
<tr>
<td><strong>Feeding route</strong></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>6 (40)</td>
</tr>
<tr>
<td>PEG</td>
<td>8 (53.33)</td>
</tr>
<tr>
<td>PEJ</td>
<td>1 (6.67)</td>
</tr>
<tr>
<td><strong>Number of regular medications; mean (SD)</strong></td>
<td>6.64 (3.57)</td>
</tr>
</tbody>
</table>
Table 4.3. Cytokine values (pg/mL) at baseline and following stimulation with 1uL lipopolysaccharide (LPS; 10ng/ml) for every 100uL of peripheral blood for 1 hour. Whole blood samples were taken from healthy controls (n=14) and children with Severe Neurological Impairment (SNI; n=15). Serum was isolated and cytokines were analysed by Enzyme Linked Immunosorbent Assay. Data are displayed as Median [95%CI] and were analysed using the Kruskal-Wallis test. A p value ≤0.05 was considered significant. * represents a p value ≤0.05; ** represents a p value ≤0.01; *** represents a p value ≤0.001; **** represents a p value ≤0.0001; SNI, Severe Neurological Impairment; Veh, Vehicle; aBaseline cytokine levels between controls and SNI; bControl cytokine levels before and after stimulation with LPS; cSNI cytokine levels before and after stimulation with LPS; dCytokine levels in control and SNI groups following LPS stimulation; EPO, Erythropoeitin; GM-CSF, Granulocyte-Monocyte Colony Stimulating Factor; INF-γ, Interferon Gamma; IL, Interleukin; TNF, Tumour Necrosis Factor; VEGF, Vascular Endothelial Growth Factor.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Control</th>
<th>SNI</th>
<th>p&lt;sub&gt;a&lt;/sub&gt;</th>
<th>p&lt;sub&gt;b&lt;/sub&gt;</th>
<th>p&lt;sub&gt;c&lt;/sub&gt;</th>
<th>p&lt;sub&gt;d&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>Veh</td>
<td>LPS</td>
<td>0.11 [0.08, 0.15]</td>
<td>0.375 [0.12, 0.82]</td>
<td>&gt;0.99</td>
<td>0.04*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>9.925 [6.62, 14.89]</td>
<td>11.17 [6.8, 20.81]</td>
<td>&gt;0.99</td>
<td>0.84</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.36 [0.25, 0.75]</td>
<td>0.34 [0.29, 0.55]</td>
<td>&gt;0.99</td>
<td>0.02*</td>
<td>0.0005***</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>IL-18</td>
<td>597 [464.2, 1003]</td>
<td>526.6 [343.3, 666.5]</td>
<td>0.37</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>175.9 [125.9, 364.3]</td>
<td>196.9 [162, 268.7]</td>
<td>&gt;0.99</td>
<td>&lt;0.0001****</td>
<td>0.0007***</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.17 [0.05, 0.52]</td>
<td>0.26 [0.12, 0.59]</td>
<td>1.17 [0.7, 1.52]</td>
<td>&gt;0.99</td>
<td>0.0008***</td>
<td>0.0023**</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.54 [0.33, 2.74]</td>
<td>1.02 [0.62, 1.62]</td>
<td>6.34 [3.74, 10.97]</td>
<td>&gt;0.99</td>
<td>0.0002***</td>
<td>0.002**</td>
</tr>
<tr>
<td>IL-8</td>
<td>3.71 [2.54, 5.49]</td>
<td>5.16 [3.41, 10.53]</td>
<td>238.6 [172.9, 367.8]</td>
<td>&gt;0.99</td>
<td>0.001***</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3.265 [2.2, 9.19]</td>
<td>2.83 [2.4, 4.06]</td>
<td>378.2 [288.2, 537.6]</td>
<td>&gt;0.99</td>
<td>0.001***</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>TNF-β</td>
<td>0.42 [0.21, 0.77]</td>
<td>0.825 [0.3, 1.74]</td>
<td>0.43 [0.11, 1.5]</td>
<td>&gt;0.99</td>
<td>0.58</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>EPO</td>
<td>61.39 [47.54,113.9]</td>
<td>103.8 [71.34, 149.5]</td>
<td>144.1 [96.2, 215.3]</td>
<td>0.60</td>
<td>&gt;0.99</td>
<td>0.71</td>
</tr>
<tr>
<td>VEGF</td>
<td>29.55 [23.11, 46.06]</td>
<td>125.7 [94.48, 153.5]</td>
<td>37.9 [29.28, 70.96]</td>
<td>&gt;0.99</td>
<td>0.001***</td>
<td>0.0004***</td>
</tr>
</tbody>
</table>
Figure 4.1. Cytokine values (pg/mL) at baseline and following stimulation for 1 hour with 1μL lipopolysaccharide (LPS; 10ng/ml) for every 100μL of peripheral blood. Whole blood samples were taken from healthy controls (Con; n=14) and children with Severe Neurological Impairment (SNI; n=15). Serum was isolated and cytokines were analysed by Enzyme Linked Immunosorbent Assay. Data are...
displayed as Median [95%CI] and were analysed using the Kruskal-Wallis test. A p value ≤0.05 was considered significant. * represents a p value ≤0.05; ** represents a p value ≤0.01; *** represents a p value ≤0.001; **** represents a p value ≤0.0001; Veh, Vehicle.
Table 4.4. Percentage change in cytokine levels following stimulation for 1 hour with 1μL lipopolysaccharide (LPS; 10ng/ml) for every 100μL of peripheral blood. Whole blood samples were taken from healthy controls (Con; n=14) and children with Severe Neurological Impairment (SNI; n=15). Serum was isolated and cytokines were analysed by Enzyme Linked Immunosorbent Assay. Data are displayed as Median [95%CI] except INF-γ, IL1-Ra, TNF-β and VEGF which are displayed as Mean (SD). Normally distributed data were analysed using the student t test while the Mann-Whitney U test was used to analyse data where normal distribution could not be assumed. A p value ≤0.05 was considered significant. * represents a p value ≤0.05; ** represents a p value ≤0.01; Veh, Vehicle; EPO, Erythropoietin; GM-CSF, Granulocyte-Monocyte Colony Stimulating Factor; INF-γ, Interferon Gamma; IL, Interleukin; TNF, Tumour Necrosis Factor; VEGF, Vascular Endothelial Growth Factor.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Control (n=14)</th>
<th>SNI (n=15)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPO</td>
<td>16.53 [7.44, 28.86]</td>
<td>36.77 [22.16, 62.42]</td>
<td>0.0068**</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>115.4 [-4.76, 816.7]</td>
<td>194.8 [9.09, 412.5]</td>
<td>0.9286</td>
</tr>
<tr>
<td>INF-γ mean(SD)</td>
<td>33.1 (±48.52)</td>
<td>40.77 (±61.01)</td>
<td>0.7293</td>
</tr>
<tr>
<td>IL-10</td>
<td>71.19 [25.64, 154.1]</td>
<td>104.9 [63.1, 186.2]</td>
<td>0.1225</td>
</tr>
<tr>
<td>IL-18</td>
<td>9.095 [3.03, 19.88]</td>
<td>18 [7.46, 26.36]</td>
<td>0.1456</td>
</tr>
<tr>
<td>IL-1ra mean(SD)</td>
<td>442.9 (±307.4)</td>
<td>318.7 (±212.7)</td>
<td>0.2142</td>
</tr>
<tr>
<td>IL-1β</td>
<td>986.7 [121.2, 2196]</td>
<td>325 [147.3, 522.2]</td>
<td>0.054</td>
</tr>
<tr>
<td>IL-6</td>
<td>1106 [541.9, 2320]</td>
<td>443.1 [216.9, 728.7]</td>
<td>0.012*</td>
</tr>
<tr>
<td>IL-8</td>
<td>2288 [1246, 3664]</td>
<td>3495 [2133, 5448]</td>
<td>0.23</td>
</tr>
<tr>
<td>TNF-α</td>
<td>10645 [2562, 14565]</td>
<td>11417 [8159, 21269]</td>
<td>0.50</td>
</tr>
<tr>
<td>TNF-β mean(SD)</td>
<td>119.9 (±177.5)</td>
<td>-1.46 (±78.29)</td>
<td>0.051</td>
</tr>
<tr>
<td>VEGF mean(SD)</td>
<td>293.7 (±144.7)</td>
<td>273.8 (±135.8)</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Figure 4.2. Percentage change in cytokine values following stimulation for 1 hour with 1uL lipopolysaccharide (LPS; 10ng/ml) for every 100uL of peripheral blood. Whole blood samples were taken from healthy controls (Con; n=14) and children with Severe Neurological Impairment (SNI; n=15). Serum was isolated and cytokines were analysed by Enzyme Linked Immunosorbent Assay. Data are displayed as Median [95%CI] except INF-γ, IL1-Ra, TNF-β and VEGF which are displayed as Mean (SD). Normally distributed data were analysed using the student t test while the Mann-Whitney U test was
used to analyse data where normal distribution could not be assumed. A p value ≤0.05 was considered significant. * represents a p value ≤0.05; ** represents a p value ≤0.01; Veh, Vehicle; EPO, Erythropoietin; GM-CSF, Granulocyte-Monocyte Colony Stimulating Factor; INF-γ, Interferon Gamma; IL, Interleukin; TNF, Tumour Necrosis Factor; VEGF, Vascular Endothelial Growth Factor.
4.4.3 *Correlation of cytokines with other markers of health*

Cytokine responses to LPS in the SNI group were correlated with a number of outcome markers: total number of regular medications; number of antiepileptic drugs (AEDs); number of regular medications used to treat disorders of the gastrointestinal system (GI medications); number of significant infections in the past year; number of hospital admissions in the past year; and score on the Caregiver Priorities and Child Health Index of Life with Disabilities (CPCHILD) questionnaire.

Total number of medications, number of GI medications and number of hospital admissions in the past year did not correlate with responsiveness of any of the cytokines which were analysed. Number of AEDs correlated positively with responsiveness of IL-1ra ($r=0.6217$, 95% CI $0.1443$ to $0.8644$, $p=0.0155$) and negatively with TNF-$\alpha$ ($r=0.5212$, 95% CI $-0.0046$ to $-0.8212$, $p=0.0487$). A significant negative correlation was found between number of infections within the last year and response of IL-18 to LPS ($r=-0.5844$, 95% CI $-0.8487$ to $-0.0864$, $p=0.0253$). Sleep score, as measured on the Children's Sleep Habit Questionnaire (CSHQ), was negatively correlated with EPO responsiveness ($r=-0.6331$, 95%CI $-0.8976$ to $-0.033$, $p=0.0413$).

Logistic regression was used to evaluate whether there was an association between cytokine responses and independent categorical variables, namely: sex, requirement for prophylactic antibiotics, enteral feeding or respiratory support. Requirement for enteral feeding was associated with a lower response of IL-10 (OR $0.87$, 95%CI $0.67$ to $0.99$, $p=0.0342$) and EPO (OR $0.8586$, 95%CI $0.68$ to $0.96$, $p=0.0012$) to LPS. Use of prophylactic antibiotics was associated with lower IL-10 (OR $0.83$, 95% CI $0.63$ to $0.95$, $p=0.0011$) responsiveness. Requirement for respiratory support was associated with lower VEGF (OR $0.98$, 95% CI $0.959$ to $0.996$, $p=0.0079$).

4.5 *Discussion*

We have shown alterations in pro and anti-inflammatory cytokines in children with SNI. This suggests an underlying abnormal inflammatory state which may have important effects on morbidity and mortality in this population. In particular, this may result in significant ramifications for immune function. There may also be a role for altered inflammatory responses in leading to tertiary neurological injury.

The possibility of altered inflammatory responses in children with SNI was suggested by findings in children with NE. Research in neonates with NE has shown systemic inflammatory responses with alterations in several cytokines. Sweetman et al reported on 94 neonates with moderate/severe NE(432). They showed elevated IL-8 and GM-CSF in NE compared to controls. Raised IL-8 on the first day of life (DoL) correlated with mortality, while GM-CSF on DoL 7 correlated negatively with neurodevelopmental
outcome. In term infants exposed to perinatal asphyxia (PA), EPO and VEGF are significantly altered. Infants exposed to PA had significantly raised EPO and lower levels of VEGF compared to controls. Raised EPO was associated with severe abnormalities on magnetic resonance imaging (MRI) and mortality (253). Further evidence of the importance of systemic inflammation in neurological injury is suggested by the fact that proinflammatory cytokine polymorphisms are associated with the development of CP (436).

Importantly, cytokine dysregulation is now known to persist in school-age children following NE (n=37) (323). Baseline levels of GM-CSF, TNF-β, IL-2, IL-6 and IL-8 were higher in children post-NE and GM-CSF and IL-8 responded more vigorously to LPS stimulation. LPS hyporesponsiveness was seen in EPO and TNF-β (323). Similarly in school-age children with CP (n=12), cytokine dysregulation was noted, although the pattern of altered responses was slightly different to the aforementioned NE group, with LPS hyporesponsiveness seen in IL-1α, IL-1β, IL-2 and IL-6 (429). This may reflect the more heterogeneous nature of CP aetiology in the latter study. Our findings support the conclusions of both studies by confirming that children with neurological impairment have cytokine dysregulation.

None of the cytokines measured were significantly different at baseline compared to the control group. This is similar to the findings by Zareen et al in the CP population (429), although they reported a higher EPO at baseline in the children with CP. However, our cohort of children did demonstrate a significantly stronger EPO response to LPS stimulation than the control population. EPO is an acidic glycoprotein essential to the production of red blood cells. The kidneys are the main source of production in response to reduction in the partial pressure of oxygen (pO2). Subsequently, however, EPO expression has been noted in several other organs including the liver, brain, spleen, and lung (437). Non-erythropoietic effects of EPO have been suggested in immune modulation; regulation of vascular tone in response to an acute ischaemic event; muscle development and remodelling; metabolic homeostasis; and bone formation and homeostasis (438). Its role in immune modulation and its anti-apoptotic effects, in particular, have led to much research into potential roles in neuroprotection and treatment of sepsis (439-442). A meta-analysis of EPO in hypoxic-ischaemic encephalopathy (HIE) suggested a reduction in risk of brain injury, CP, and cognitive impairment (443). The HEAL randomised controlled trial of high dose EPO for perinatal asphyxia and encephalopathy in term infants is currently ongoing but early phase I/II trials have shown promise (444). On the other hand, in preterm infants, a large randomised double-blind trial (the PENUT trial) found that EPO did not reduce the occurrence of severe neurodevelopmenetal impairment or death at 2 years (445). Other studies have shown that elevated EPO concentrations are associated with poorer neurodevelopmental outcome and increased mortality in preterm infants and
those with NE (253, 446). It is, therefore, difficult to determine whether the increased LPS-responsiveness of EPO in our patients is advantageous or deleterious. Most likely, the answer to this question is complex and depends on the site and timing of its production. What is clear is that there is an underlying alteration in the inflammatory process in children with SNI.

Our results suggest that children with SNI have a less robust response in GM-CSF than controls. GM-CSF was originally thought of as a protein which could produce granulocytes and macrophages from myeloid precursor cells(447). Sources of GM-CSF include epithelial cells, endothelial cells, fibroblasts, and haematopoietic cells. It promotes survival of macrophages, neutrophils, and eosinophils. However, in the steady state, lack of GM-CSF does not appear to have a significant impact on myeloid system (447). One of the most significant roles of GM-CSF is in inducing emergency haematopoiesis on exposure to inflammatory stimuli, including endotoxins such as LPS. In animal models, the most significant issue in GM-CSF deficient mice is the compromise of alveolar macrophage maturation. This increases susceptibility to certain pulmonary infections, including Group B Streptococcus (448), Pneumocystis carinii(449) and Pseudomonas aeruginosa (450, 451). In humans, GM-CSF has been shown to modulate pulmonary resistance to Influenza A, with a reduction in GM-CSF and resulting reduction in alveolar macrophages leading to increased mortality (452, 453). Children with recurrent respiratory tract infections have been shown to have reduced GM-CSF compared to those presenting with a single respiratory tract infection (454). Pulmonary infections are one of the leading causes of morbidity and mortality in children with neurodevelopmental disorders such as CP, which begs the question whether a lack of a significant response of GM-CSF to LPS, as we have shown in children with SNI, may be a contributory factor. The importance of this lies in the potential therapeutic effect of administering GM-CSF to reduce mortality(452, 453, 455, 456). In previous studies, GM-CSF has been found to be elevated on DoL 1 in infants with NE(431), and lower on DoL 2 and 3, the latter correlating with abnormalities on MRI brain(457). The development of CP has been linked with lower GM-CSF levels in cord blood of preterm infants(458). In contrast to our findings, Zareen et al did not find significantly different GM-CSF responses to LPS in children with CP compared to controls, although the participants in this study are not directly comparable to the children with SNI who participated in our study. Further research is warranted including larger numbers of children to confirm our findings and to assess the potential of GM-CSF as a therapeutic target in improving outcomes, in particular, with regards to respiratory tract infections in this population. It is also known that neutrophil apoptosis is significantly delayed by GM-CSF in adults, but not in neonates(459). Research in older children is required to distinguish whether the responses of their neutrophils to GM-CSF follows the pattern of adults or neonates, or an intermediate response. This could have important
therapeutic repercussions related to the use of GM-CSF in the treatment of sepsis in children, especially those with SNI, who we have shown to have a less robust GM-CSF response to LPS than controls.

We have also demonstrated significant hyporesponsiveness of IL-6 to stimulation with LPS. In both controls and children with SNI, there was a significant rise of IL-6 following endotoxin exposure compared to baseline levels. However, in the SNI population the relative rise was significantly less than that of controls. This is in keeping with the findings of Zareen et al that children with CP have IL-6 hyporesponsiveness(429). A systematic review of inflammatory biomarkers in children with CP found that higher levels of IL-6 are associated with abnormal neurological outcome(460) and IL-6 polymorphisms have been shown to increase the risk of developing CP(461, 462). It is possible that altered inflammation in early life may have a programming effect and alter inflammatory responses later in life(463). IL-6 is a significant component of the systemic inflammatory response to infection(464) and correlates with survival of patients with sepsis(465). Critically ill adult patients admitted to ICU have been found to have lower production of IL-6 in response to LPS. This, amongst other immune markers, may indicate an impaired inflammatory response in the early phase of critical illness(466). Cetiner et al reported a decreased IL-6 level, along with reduced TNF-α, and increased IL-10 and IL-4 in children with Down syndrome. It is proposed that this anti-inflammatory picture may explain the higher rate of respiratory tract infections in this cohort(467). IL-6 hyporesponsiveness, as seen in our study, may affect the ability of children with SNI to respond adequately to infection. Future research may focus on prospectively measuring IL-6 in these children and following outcomes such as number of infections per year, requirement for antibiotic treatment, infection-related hospitalisations, intensive care admissions related to infectious causes, sepsis, and mortality.

We correlated cytokine responses in the SNI group with several other health-related outcome measures. The CSHQ score and requirement for respiratory support were positively associated with EPO and VEGF respectively. This is not surprising considering that both cytokines are sensitive to hypoxia and have been shown to be raised in obstructive sleep apnoea(468, 469).

We have demonstrated that recurrent respiratory tract infections were negatively associated with IL-18 responses. IL-18 is a pro-inflammatory cytokine which induces INF-γ nitric oxide and reactive oxygen species in phagocytes and thus plays a role in clearance of various micro-organisms. It also activates CD8+ T cells which are involved with viral clearance. On the other hand, IL-18 hyper-responsiveness has been implicated as a contributory factor to the cytokine storm and increased mortality in conditions such as sepsis and Coronavirus Disease 2019 (COVID-19)(470). Further research is required to
determine the role of IL-18 in respiratory tract infections in children with SNI and whether it may prove useful as a therapeutic target.

Use of prophylactic antibiotics was associated with IL-10 levels. Azithromycin was the most commonly used prophylactic antibiotic in our population of children with SNI. Macrolide antibiotics are known to have bacteriostatic activity but also exert an effect through their immunomodulatory properties\(^{(471)}\). IL-10 is a predominantly anti-inflammatory cytokine, and it is probable that the anti-inflammatory properties of azithromycin are, in part, exerted through modulation of IL-10 production\(^{(472)}\).

Requirement for enteral feeding, such as with percutaneous endoscopic gastrostomy (PEG) or percutaneous endoscopic jejunostomy (PEJ), was associated with lower IL-10 and EPO responsiveness. Research is lacking in this area but, in contrast to our findings, a randomised double-blind trial in adults with acute pancreatitis showed increased IL-10 levels in those administered enteral nutrition versus those who received parenteral nutrition\(^{(473)}\). In neonates, delayed enteral feeding is associated with decreased IL-10:IL-8 ratios\(^{(474)}\).

Finally, we demonstrated that increasing number of AEDs was associated with higher IL-1ra and with lower TNF-\(\alpha\). Epilepsy and seizures are known to be associated with a pro-inflammatory state\(^{(475-477)}\). The anti-inflammatory effect of AEDs on cytokines has been well demonstrated and has been proposed as one of the mechanisms of action of these drugs\(^{(475, 478)}\). IL-1ra has powerful anti-convulsant effects\(^{(479)}\) and potential as a therapeutic target in neuroprotection and seizure prevention\(^{(480)}\). Similar to our findings, TNF-\(\alpha\) was found to be decreased in response to 9 different AEDs in in-vitro studies\(^{(478)}\). IL-1ra is an anti-inflammatory cytokine, while TNF-\(\alpha\) is considered predominantly pro-inflammatory. Thus, the overall association appears to anti-inflammatory with increasing numbers of AEDs. This may be considered a beneficial side-effect of anti-epileptic medications and indeed, in several animal studies AEDs have been shown to have beneficial effects in systemic inflammatory states such as sepsis\(^{(481-486)}\).

The main limitation of this study is the small sample size. However, our findings are in keeping with previous studies in the area and serve to confirm these results. There is a significant paucity of research regarding cytokine dysregulation in children with neurodevelopmental disorders, particularly those at school-age. Despite the small number of patients included we believe our findings raise important questions regarding the altered inflammatory state in children with SNI and we hope this will prompt further larger studies in the area. Another criticism is the heterogeneity of the study population. We would argue that our population is no more heterogeneous than other cohorts of patients who are regularly grouped together for research or clinical purposes. For example, those with CP, epilepsy, and NE, have a wide variety of causes including genetic, infectious, congenital, and traumatic aetiologies. A better understanding of cytokine dysregulation
has the potential to lead to an enhanced array of therapeutic options which may ultimately lead to improved health-related outcomes.
Chapter 5 – Immune Function in Severe Neurological Impairment
5.1 Introduction

Children with Severe Neurological Impairment (SNI) have permanent disorders of the central nervous system (CNS) which result in motor impairment, cognitive impairment, and medical complexity (428). In some cases, a child with SNI may not have a unifying diagnosis. The exact proportion without a diagnosis is difficult to quantify but it has been reported that 30-50% of children with severe learning disability and 50% of children with multiple congenital anomalies do not have a definitive diagnosis (487-489). Over 13% of children referred for paediatric palliative care had a syndrome without a name (SWAN), the majority of whom had neurological issues (490). Lack of a clear diagnosis creates additional problems for these families (489). Children with SNI share the potential for dysfunction in almost every system as a comorbidity (491).

Infections, in particular those of the respiratory tract, are a major cause of morbidity and mortality in children with neurological impairment (492). This is most marked in those with the greatest motor impairment or the greatest total disability score (492), as would be seen in those with SNI. In a retrospective analysis of post-operative complications of appendectomy (n=1250), Dhiman et al have described significantly greater odds of sepsis/organ failure, operation-related infection, pneumonia, and urinary tract infection in children with cerebral palsy (CP) compared to those without (261). Despite the higher burden of neurological impairment, there is relatively little data from low- and middle-income countries on causes of death. However, meningitis, pneumonia, and malaria account for a significant proportion of premature mortality amongst children with neurological impairments in these countries (492-494). The higher risk of infection in the population of children with neurodisability is likely multi-factorial including respiratory muscle weakness, poor clearance of secretions and aspiration contributing to respiratory tract infections (52). However, one cannot discount the potential role for immune dysfunction in the development of significant infection-related morbidity and mortality.

Relatively little research exists on leukocyte function in children with neurodevelopmental disorders. Previous research has shown subclinical immunologic abnormalities with γδT cell expansion in patients with neurological disorders, particularly CP (495). In addition, Kadhim et al reported that markers of T-lymphocyte activation are upregulated in cerebral palsy (496). More recently, Taher et al have demonstrated alterations in T and B cell proportions, distributions, and functions in children with neonatal encephalopathy (NE) and in children with CP (497). With regard to markers of granulocyte function, toll-like receptor (TLR)-4 has been shown to be increased in preterm children with CP at school-age (126). In children with autism spectrum disorder, TLR4 expression is altered and proposed to contribute to immune dysfunction in these children (498). Cytokine dysregulation is seen in childhood post NE and also in children with CP (323, 429).
Another vital component of the innate immune response to infection is the inflammasome, of which the nucleotide-binding and oligomerization domain (NOD)-like receptor family, NOD-like receptor protein 3 (NLRP3) is the most widely studied (499). The NLRP3 inflammasome has been implicated in numerous disease states (430), including neurological injury (500), and altered activation of the inflammasome has been shown to persist in childhood following NE (430). It is expressed in neutrophils, monocytes, and lymphocytes (501). The NLRP3 inflammasome consists of a sensor (NLRP3), an adaptor (Apoptosis-associated speck-like protein containing a CARD; ASC) and an effector (Caspase-1), the activation of which leads to secretion of the pro-inflammatory cytokines, IL1β and IL18 (499). NLRP3 inflammasome assembly also causes cleavage of gasdermin D (GSDMD), triggering pyroptosis (a highly inflammatory form of programmed cell death) (499). GSDMD exhibits bactericidal activity, contributing to the innate immune response to infection (499).

The aforementioned observations regarding immune function and the inflammasome in children with neurological impairment, as well as their predisposition to infection-related morbidity and mortality, raise the possibility of clinically relevant immune dysfunction in this population. We therefore sought to examine leukocyte proportions and function, as well as inflammasome activation, in children with SNI compared with age and sex-matched controls.

5.2 Hypothesis

In response to stimulation with lipopolysaccharide (LPS), children with SNI have hyperresponsive markers of activation on neutrophils, monocytes and lymphocytes, and up-regulation of the NLRP3 inflammasome, compared to healthy controls.

5.3 Aims

To describe neutrophil, monocyte and lymphocyte proportions and activation, at baseline and in response to stimulation with lipopolysaccharide, in children with SNI compared to a control group of healthy children.

Deliverables:

1. Examine differences in lymphocyte subsets, at baseline, between children with SNI and controls.
2. Evaluate the in-vitro effects of LPS on monocyte and neutrophil activation in controls compared to children with SNI.
3. Evaluate the expression of the NLRP3, ASC and IL1β genes at baseline and in response to stimulation with LPS in controls versus children with SNI.
5.4 Results

5.4.1 Participant characteristics

Details of the recruitment of participants can be found in chapter 2, section 4.1. Controls and children with SNI were recruited from the outpatient department of Children’s Health Ireland (CHI) at Tallaght. Children with SNI had permanent disorders of the CNS resulting in motor impairment, cognitive impairment and medical complexity requiring much assistance with activities of daily living (428). Details of their diagnoses are included in (Table 5.1). Controls were healthy children, having phlebotomy performed for reasons unrelated to acute infection/inflammation or chronic illness.

Fourteen controls, 7 of whom were male, had a mean age of 8.1±4.5 years.
Fourteen children with SNI, 7 of whom were male, had a mean age of 11.4±4.8 years. There were no significant differences in age (p=0.07) or sex (p>0.9999) between the 2 groups.

5.4.2 Lymphocytes in children with SNI compared to controls

Whole blood samples were prepared, and flow cytometry was used to quantify lymphocyte subset proportions, as described in chapter 2. All data are presented as medians with 95% confidence intervals, except for B cells which were normally distributed and, therefore, are expressed as means with standard deviation.

T cells, as a proportion of total lymphocytes, were found to be significantly lower at baseline in children with SNI compared to controls (67.2%, 95%CI 54.91-78.49 vs 78.91%, 95%CI 71.43-81.11 respectively; p=0.02 (Figure 5.1A). CD4+, γδ1+ and γδ2+ T cells were not significantly different between the two groups (Table 5.2 and Figure 5.1B, D & E). However, baseline CD8+ T cells were lower in those with SNI than controls (p=0.0031)(Table 5.2 and Figure 5.1C).

B cells, at baseline, were not significantly different between the 2 groups (Controls vs SNI: 13.3±3.48 vs 18.44±10.2; p=0.09)(Figure 5.1F). Similarly, Natural Killer (NK) cells were not different in children with SNI when compared with control children (Controls vs SNI: 9.807%, 95%CI 4.94-11.76 vs 13.04, 95%CI 6.53-19.91; p=0.16)(Figure 5.1G).
Table 5.1. Details of diagnoses of children with Severe Neurological Impairment (SNI). CP, Cerebral Palsy; CASK, Calcium/Calmodulin Dependent Serine Protein Kinase.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>9 (64.3%)</td>
</tr>
<tr>
<td>-Dyskinetic</td>
<td>4</td>
</tr>
<tr>
<td>-Spastic</td>
<td>3</td>
</tr>
<tr>
<td>-Mixed</td>
<td>2</td>
</tr>
<tr>
<td>Wolf Hirschhorn Syndrome</td>
<td>2 (14.3%)</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>2 (14.3%)</td>
</tr>
<tr>
<td>CASK mutation</td>
<td>1 (7.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aetiology of CP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal Encephalopathy</td>
<td>3</td>
</tr>
<tr>
<td>Congenital brain malformation</td>
<td>1</td>
</tr>
<tr>
<td>Infection</td>
<td>2</td>
</tr>
<tr>
<td>Prematurity</td>
<td>2</td>
</tr>
<tr>
<td>Genetic</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 5.2. T cell subset proportions as a percentage of total T cells. Whole blood samples were collected from controls (n=14) and children with Severe Neurological Impairment (SNI; n=14). Samples were processed for flow cytometry CI, Confidence Interval. **p<0.01

<table>
<thead>
<tr>
<th>T cell subset</th>
<th>Control</th>
<th>SNI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>95% CI</td>
<td>Median</td>
</tr>
<tr>
<td>CD4+</td>
<td>49.05</td>
<td>45.70-53.60</td>
<td>56.20</td>
</tr>
<tr>
<td>CD8+</td>
<td>38.00</td>
<td>35.50-40.00</td>
<td>33.30</td>
</tr>
<tr>
<td>γδ1+</td>
<td>2.65</td>
<td>1.48-4.30</td>
<td>1.32</td>
</tr>
<tr>
<td>γδ2+</td>
<td>3.25</td>
<td>1.45-6.24</td>
<td>3.55</td>
</tr>
</tbody>
</table>
Figure 5.1. Proportion of T cells and CD8+ cells as a percentage of the total lymphocyte count are significantly lower in children with SNI compared to controls.

Samples were processed for flow cytometry. Values are expressed as percentages of total lymphocytes or T cells. Mann-Whitney test (median, 95%CI). Control (Con, n=14); Severe Neurological Impairment (SNI, n=14); NK, Natural Killer cells; *p≤0.05; **p≤0.01.
5.4.3 Effect of LPS endotoxin on Neutrophils

Values for CD66b, TLR4 and CD11b are expressed as mean channel fluorescence (MCF). MCF is the average fluorescence intensity of cell populations following the binding of fluorescent-labelled antibodies. There was no difference in CD66b expression in either cohort at baseline (p=0.33)(Table 5.3 & Figure 5.2B). Both controls and children with SNI responded to LPS stimulation with significantly greater CD66b expression than at baseline. In controls, CD66b expression increased from 1888 at baseline to 10048 following LPS stimulation (p=0.0004)(Table 5.3 & Figure 5.2B). In the SNI population, CD66b expression increased from 5711 to 14334 following LPS endotoxin exposure (p=0.0027)(Table 5.3 & Figure 5.2B). Although both cohorts had significant increases in CD66b expression following LPS, children with SNI were relatively hyporesponsive, with a median percentage increase of 138.70% compared to 388.30% in the controls (p=0.0017)(Table 5.4 & Figure 5.3A).

Neutrophil TLR4 was not significantly different at baseline between the control and SNI groups (p>0.99)(Table 5.3 & Figure 5.2C). TLR4 expression did not significantly increase following LPS stimulation in either group and there was no significant difference in expression following stimulation (p=0.96)(Table 5.3 & Figure 5.2C). There was no difference in percentage change of neutrophil TLR4 expression following LPS stimulation in either group (p=0.73)(Table 5.4 & Figure 5.3B).

Neutrophil CD11b expression at baseline was not significantly different between controls and children with SNI (p>0.99)(Table 5.3 & Figure 5.2D). In both cohorts, expression of CD11b increased with LPS stimulation. In controls, CD11b expression increased from a median of 5891 to 27261 (p=0.0006)(Table 5.3 & Figure 5.2D). In the SNI group, expression of CD11b increased from a baseline of 4658 to 32435 following LPS stimulation (p=0.0001)(Table 5.3 & Figure 5.2D). There was no significant difference in CD11b expression post-LPS stimulation between the groups (p=0.99)(Table 5.3 & Figure 5.2D). The percentage change in neutrophil CD11b was not significantly different between controls and those with SNI (p=0.38)(Table 5.4 & Figure 5.3C).
Figure 5.2. Neutrophil proportions and markers of activation at baseline and following 1uL lipopolysaccharide (LPS; 10ng/ml) stimulation.

Whole blood samples were processed for flow cytometry and expression of CD66b, TLR4 and CD11b on neutrophils was quantified. Neutrophil proportions are expressed as a percentage of total granulocyte numbers; values for CD66b, TLR4 and CD11b are expressed as Mean Channel Fluorescence (MCF). Kruskal Wallis test (median, 95%CI); Control (Con, n=14); Severe Neurological Impairment (SNI, n=14); Vehicle (Veh); **p<0.01; ***p<0.001; ****p<0.0001; (A) Proportion of neutrophils in total granulocytes; (B) Neutrophil CD66b expression; (C) Neutrophil TLR4 expression; (D) Neutrophil CD11b expression.
Figure 5.3. Percentage change in expression of markers of activation in neutrophils following 1uL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour.

Whole blood samples were processed for flow cytometry and expression of CD66b, TLR4 and CD11b on neutrophils was quantified. Values are shown as percentage change in expression of CD66b, TLR4 and CD11b following incubation with LPS (10ng/ml) for 1 hour. Mann-Whitney test (median, 95%CI). Control (Con, n=14); Severe Neurological Impairment (SNI, n=14); **p≤0.01; (A) change in neutrophil CD66b; (B) change in neutrophil TLR4; (C) change in neutrophil CD11b.
5.4.4 Effect of LPS endotoxin on Monocytes

Monocyte proportions at baseline were noted to be significantly lower in children with SNI when compared to controls (p=0.0002). There was no significant change in total monocyte proportions following LPS endotoxin stimulation in either group (Table 5.3 & Figure 5.4A).

There was no significant difference in monocyte TLR4 expression, at baseline, in either group (p>0.9999)(Table 5.3 & Figure 5.4B). Similarly, there was no significant difference in TLR4 expression between controls and children with SNI following LPS endotoxin exposure (p>0.9999)(Table 5.3 & Figure 5.4B). However, there was a significant difference in the percentage change in monocyte TLR4 expression with LPS stimulation (p=0.04) with the SNI cohort showing hyper-responsiveness in this case (Table 5.4 & Figure 5.5A).

At baseline, monocyte CD11b expression was no different between controls and children with SNI (p=0.55)(Table 5.3 & Figure 5.4C). In both groups, CD11b significantly increased with LPS exposure (p<0.0001)(Table 5.3 & Figure 5.4C). Monocyte CD11b expression was not significantly different following LPS stimulation between the groups (p=0.17)(Table 5.3 & Figure 5.4C). Regarding percentage change in CD11b, there was no statistically significant difference between the groups (p=0.63)(Table 5.4 & Figure 5.5B).

5.4.5 Effect of LPS endotoxin on Monocyte subsets

Monocytes were divided into their subsets based on CD14 and CD16 positivity, namely: Classical (CD14+/CD16 dim), Intermediate (CD14+/CD16+), and Non-classical (CD14 dim/CD16+). Proportions of classical monocytes did not differ between the groups either before (p>0.9999) or after stimulation with LPS (p>0.9999)(Table 5.3 & Figure 5.6).

Proportions of intermediate monocytes were not significantly different between the control and SNI group at baseline (p=0.45) or following LPS exposure (p>0.9999)(Table 5.3 & Figure 5.6). However, in the control group, intermediate monocyte proportions reduced from 7.38 to 3.995 after LPS exposure (p=0.04)(Table 5.3 & Figure 5.6). There was no such reduction in the cohort of children with SNI.

Non-classical monocytes did not differ between the groups at baseline (p=0.20) or following stimulation with LPS (p>0.99)(Table 5.3 & Figure 5.6). Neither group showed a significant difference in non-classical monocyte proportions after LPS exposure.
Figure 5.4. Monocyte proportions and markers of activation at baseline and following LPS endotoxin exposure for 1 hour.

Whole blood samples were processed for flow cytometry and expression of TLR4 and CD11b on monocytes was quantified. Proportion of monocytes are expressed as a percentage (%) of total granulocytes. Values for TLR4 and CD11b are expressed as Mean Channel Fluorescence (MCF). Kruskal-Wallis test (median, 95%CI). Control (Con, n=14); Severe Neurological Impairment (SNI, n=14); Vehicle (Veh); **p<0.01; ***p<0.001; ****p<0.0001; (A) Proportion of monocytes in total granulocytes; (B) Monocyte TLR4 expression (Con n=11; SNI n=13); (C) Monocyte CD11b expression.
Whole blood samples were processed for flow cytometry and expression of TLR4 and CD11b on monocytes was quantified. Values are shown as percentage change in expression of TLR4 and CD11b following incubation with LPS (10ng/ml) for 1 hour. Control (Con, n=14); Mann-Whitney U test (median, 95% CI); Severe Neurological Impairment (SNI, n=14); *p<0.05; (A) change in monocyte TLR4; (B) change in monocyte CD11b.

Figure 5.5. Percentage change in expression of markers of activation in monocytes following 1uL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour.
5.4.5.1 Effect of LPS endotoxin on TLR4 expression in monocyte subsets

Expression of TLR4 in classical monocytes did not differ at baseline (p>0.99) or following LPS exposure (p>0.99) between controls and children with SNI (Table 5.3 & Figure 5.7). There was a relative hyperresponsiveness of classical cell TLR4 on endotoxin exposure (p=0.03)(Table 5.4 & Figure 5.8).

Intermediate monocytes did not show a significant difference in TLR4 expression at baseline (p>0.99) or following stimulation with LPS (p=0.70)(Table 5.3 & Figure 5.7). There was no difference in percentage change of intermediate cell TLR4 expression following LPS stimulation between controls and those with SNI (p=0.88)(Table 5.4 & Figure 5.8).

Finally, in non-classical monocytes, when comparing controls to children with SNI, there was no difference in TLR4 expression before (p>0.99) or after endotoxin exposure (p>0.99)(Table 5.3 & Figure 5.7). Similarly, percentage change in TLR4 expression in non-classical cells was not significantly different between groups (p=0.29)(Table 5.4 & Figure 5.8).

5.4.5.2 Effect of LPS endotoxin on CD11b expression in monocyte subsets

CD11b expression on classical monocytes was not different at baseline (p>0.9999) or following LPS exposure (p>0.9999) between groups. Both paediatric controls and children with SNI responded similarly to LPS stimulation (p=0.0005 and p=0.0006 respectively)(Table 5.3 & Figure 5.9). There was no difference in the percentage change in CD11b expression on LPS exposure (p=0.63)(Table 5.4 & Figure 5.10).

In intermediate monocytes, CD11b expression was not different at baseline (p=0.91) or following stimulation with LPS (p>0.99) when comparing the control and SNI groups. Both cohorts mounted a similar response to LPS exposure (p=0.0032 and p=0.0097)(Table 5.3 & Figure 5.9). There was no difference in percentage change of intermediate monocyte CD11b between controls and children with SNI (p=0.95)(Table 5.4 & Figure 5.10).

Non-classical monocytes did not show a significantly different CD11b expression between cohorts at baseline (p=0.92) or following endotoxin exposure (p=0.9999)(Table 5.3 & Figure 5.9). Percentage change of CD11b expression following LPS was not different between groups (p=0.29)(Table 5.4 & Figure 5.10).
Figure 5.6. Monocyte subsets as proportions of total monocytes, before and after 1uL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour.

Whole blood samples were processed for flow cytometry. Proportion of monocyte subsets are expressed as a percentage (%) of total monocytes. Kruskal-Wallis test (median, 95%CI). Control (Con, n=14); Severe Neurological Impairment (SNI, n=14); *p≤0.05.
Figure 5.7. TLR4 expression on monocyte subsets at baseline and following 1uL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour.

Samples were processed for flow cytometry and expression of TLR4 monocyte subsets was quantified. Values for TLR4 are expressed as Mean Channel Fluorescence (MCF). Kruskal-Wallis test (median, 95%CI). Control (Con, n=11); Severe Neurological Impairment (SNI, n=13); Vehicle (Veh).
Figure 5.8. Percentage change in TLR4 in monocyte subsets following 1μL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour.

Samples were processed for flow cytometry and expression of TLR4 on monocyte subsets was quantified. Values are shown as percentage change in expression of TLR4 following incubation with LPS (10ng/ml) for 1 hour. Mann-Whitney test (median, 95%CI); Control (Con, n=11); Severe Neurological Impairment (SNI, n=13); *p≤0.05.
Figure 5.9. CD11b expression on monocyte subsets at baseline and following 1μL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour.

Whole blood samples were processed for flow cytometry and expression of TLR4 monocyte subsets was quantified. Values for TLR4 are expressed as Mean Channel Fluorescence (MCF). Kruskal-Wallis test (median, 95%CI). Control (Con, n=14); Severe Neurological Impairment (SNI, n=14); Vehicle (Veh); **p<0.01; ***p<0.001.
Whole blood samples were processed for flow cytometry and expression of CD11b on monocyte subsets was quantified. Values are shown as percentage change in expression of CD11b following incubation with LPS (10ng/ml) for 1 hour. Mann-Whitney test (median, 95%CI); Control (Con, n=14); Severe Neurological Impairment (SNI, n=14).
Table 5.3. Proportions of neutrophils and monocytes and their expression of CD66b, TLR4 and CD11b at baseline and following 1uL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour. P-values in bold type are significant at the level of p≤0.05; *Baseline cell proportions or Mean Channel Fluorescence (MCF) between controls and SNI; †Control cell proportions or MCF before and after stimulation with LPS; ‡SNI cell proportions or MCF before and after stimulation with LPS; §Cell proportions or MCF in control and SNI groups following LPS stimulation.

<table>
<thead>
<tr>
<th></th>
<th>Control vehicle</th>
<th>SNI Vehicle</th>
<th>Control LPS</th>
<th>SNI LPS</th>
<th>p^a</th>
<th>p^b</th>
<th>p^c</th>
<th>p^d</th>
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<tr>
<td><strong>Neutrophil</strong></td>
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<tr>
<td>Proportion</td>
<td>80.45 [95%CI 14.60-85.90]</td>
<td>61.30 [95%CI 53.90-76.10]</td>
<td>66.60 [95%CI 51.50-78.40]</td>
<td>68.00 [95%CI 50.60-76.60]</td>
<td>0.2316</td>
<td>0.5054</td>
<td>&gt;0.9999</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>CD66b</td>
<td>1888 [95%CI 858-6287]</td>
<td>5711 [95%CI 4473-9445]</td>
<td>10048 [95%CI 10041-20031]</td>
<td>14334 [95%CI 10041-20031]</td>
<td>0.3265</td>
<td>0.0004</td>
<td>0.0027</td>
<td>0.9046</td>
</tr>
<tr>
<td>TLR4</td>
<td>636 [95%CI 487-745]</td>
<td>932 [95%CI 197-9731]</td>
<td>538 [95%CI 367-720]</td>
<td>1126 [95%CI 244-4808]</td>
<td>&gt;0.9999</td>
<td>&gt;0.9999</td>
<td>&gt;0.9999</td>
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</tr>
<tr>
<td>CD11b</td>
<td>5891, 95%CI 2761-9029</td>
<td>4658, 95%CI 999-11929</td>
<td>27261 [95%CI 14201-33820]</td>
<td>32435 [95%CI 21112-50231]</td>
<td>&gt;0.9999</td>
<td>0.0006</td>
<td>&lt;0.0001</td>
<td>&gt;0.9999</td>
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<tr>
<td>Proportion</td>
<td>36.30 [95%CI 16.50-70.00]</td>
<td>77.450 [95%CI 61.90-84.10]</td>
<td>67.95 [95%CI 51.90-79.20]</td>
<td>47.10 [95%CI 25.60-62.10]</td>
<td>0.0002</td>
<td>0.6902</td>
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<td>0.3011</td>
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<td>TLR4</td>
<td>462 [95%CI 355-563]</td>
<td>440 [95%CI 255-11106]</td>
<td>424 [95%CI 358-662]</td>
<td>697 [95%CI 284-14378]</td>
<td>&gt;0.9999</td>
<td>&gt;0.9999</td>
<td>&gt;0.9999</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>CD11b</td>
<td>6755 [95%CI 3502-10778]</td>
<td>9121 [95%CI 5475-17832]</td>
<td>22781 [95%CI 15699-32860]</td>
<td>27573 [95%CI 18088-43345]</td>
<td>&gt;0.9999</td>
<td>0.0002</td>
<td>0.0004</td>
<td>&gt;0.9999</td>
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<tr>
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<tr>
<td>Proportion</td>
<td>84.30 [95%CI 69.40-91.60]</td>
<td>84.15 [95%CI 65.80-91.80]</td>
<td>90.85 [95%CI 87.20-97.80]</td>
<td>89.9 [95%CI 78.20-96.00]</td>
<td>&gt;0.9999</td>
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<td>CD11b</td>
<td>Proportion</td>
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<td>CD11b</td>
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<tr>
<td>Intermediate Monocyte</td>
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<tr>
<td>TLR4</td>
<td>460 [95% CI 357-568]</td>
<td>466 [95% CI 256-9931]</td>
<td>422 [95% CI 360-663]</td>
<td>731 [95% CI 284-14456]</td>
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<tr>
<td>CD11b</td>
<td>7810 [95% CI 4010-10854]</td>
<td>10040 [95% CI 5980-17890]</td>
<td>24980 [95% CI 16684-33208]</td>
<td>30334 [95% CI 19016-46819]</td>
<td>&gt;0.9999</td>
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<tr>
<td>Proportion</td>
<td>7.38 [95% CI 5.03-22.50]</td>
<td>5.075 [95% CI 1.95-8.70]</td>
<td>3.99 [95% CI 1.05-6.37]</td>
<td>3.06 [95% CI 0.00-5.40]</td>
<td>0.4454</td>
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<tr>
<td>Non-classical Monocyte</td>
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<tr>
<td>TLR4</td>
<td>458 [95% CI 373-588]</td>
<td>510.5 [95% CI 335-3097]</td>
<td>487.5 [95% CI 380-676]</td>
<td>743.5 [95% CI 462-3807]</td>
<td>&gt;0.9999</td>
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<tr>
<td>CD11b</td>
<td>5865 [95% CI 3330-8224]</td>
<td>9172 [95% CI 5056-13679]</td>
<td>14029 [95% CI 7542-31842]</td>
<td>18192 [95% CI 12262-26019]</td>
<td>0.9051</td>
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<tr>
<td>Proportion</td>
<td>5.34% [95% CI 1.24-7.59]</td>
<td>10.9 [95% CI 3.34-33.3]</td>
<td>4.37 [95% CI 1.31-8.76]</td>
<td>5.41 [95% CI 1.88-19]</td>
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<tr>
<td>Non-classical Monocyte</td>
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</tr>
<tr>
<td>TLR4</td>
<td>323 [95% CI 266-382]</td>
<td>249 [95% CI 194-25462]</td>
<td>272.5 [95% CI 260-413]</td>
<td>380 [95% CI 156-26019]</td>
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<tr>
<td>CD11b</td>
<td>1602, 95%CI 845-3005</td>
<td>2610, 95%CI 170-3555</td>
<td>1975, 95%CI 1382-14968</td>
<td>2682, 95%CI 2112-4259</td>
<td>0.9198</td>
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|        |        |        |        |        |        |
Table 5.4. Percentage (%) change in expression of CD66b, TLR4 and CD11b, in whole blood, following 1uL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour.

<table>
<thead>
<tr>
<th></th>
<th>Controls - % change</th>
<th>SNI - % change</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td><strong>Neutrophil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD66b</td>
<td>388.30 [95%CI 79.41-643.90]</td>
<td>138.70 [95%CI 79.41-643.90]</td>
<td><strong>0.0017</strong></td>
</tr>
<tr>
<td>TLR4</td>
<td>0.82 [95%CI -43.63-24.51]</td>
<td>11.83 [95%CI -25.28-23.86]</td>
<td>0.7283</td>
</tr>
<tr>
<td>CD11b</td>
<td>304.90 [95%CI 203.90-551.50]</td>
<td>487.70 [95%CI 153.40-381.1]</td>
<td>0.3761</td>
</tr>
<tr>
<td><strong>Monocyte</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR4</td>
<td>6.48 [95%CI -18.18-20.00]</td>
<td>38.62 [95%CI -7.90-79.29]</td>
<td><strong>0.0410</strong></td>
</tr>
<tr>
<td>CD11b</td>
<td>243.30 [95%CI 139.10-456.80]</td>
<td>197.60 [95%CI 38.34-563.80]</td>
<td>0.6347</td>
</tr>
<tr>
<td><strong>Classical monocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR4</td>
<td>3.91 [95%CI -12.05-18.50]</td>
<td>44.40 [95% CI -1.82-81.36]</td>
<td><strong>0.0332</strong></td>
</tr>
<tr>
<td>CD11b</td>
<td>200 [95%CI 126.90-447.40]</td>
<td>169.80 [95%CI 54.38-58]</td>
<td>0.6347</td>
</tr>
<tr>
<td><strong>Intermediate monocytes</strong></td>
<td></td>
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</tr>
<tr>
<td>TLR4</td>
<td>5.64 [95%CI -4.82-23.86]</td>
<td>9.44 [95%CI -46.78-66.49]</td>
<td>0.8793</td>
</tr>
<tr>
<td>CD11b</td>
<td>126.80 [95%CI 67.04-450.70]</td>
<td>183.70 [95%CI 40.01-328.90]</td>
<td>0.9547</td>
</tr>
<tr>
<td><strong>Non-classical monocytes</strong></td>
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<td></td>
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</tr>
<tr>
<td>TLR4</td>
<td>-3.44 [95%CI -25.50-8.12]</td>
<td>8.69 [95%CI -31.07-20.94]</td>
<td>0.2945</td>
</tr>
<tr>
<td>CD11b</td>
<td>56.98 [95%CI -5.40-223.80]</td>
<td>37.15 [95%CI -4.85-72.03]</td>
<td>0.2945</td>
</tr>
</tbody>
</table>
5.4.6 *Inflammasome*

The *NLRP3* inflammasome was evaluated in a convenience sample of 6 consecutive children with CP and compared with healthy controls (n=14). There was a significantly higher *NLRP3* gene expression at baseline in children with SNI compared to controls (p=0.02). On exposure to LPS, *NLRP3* expression was significantly upregulated in controls (p<0.0001) but not in the SNI cohort (p=0.50).

Expression of *ASC* was not significantly different at baseline (p=0.33) or following treatment with LPS (p=0.99) between controls and children with SNI. *ASC* gene expression did not significantly increase following LPS exposure in either group (Control vehicle vs LPS, SNI vehicle vs LPS: p=0.95, p=0.82).

Expression of the *IL1β* gene was not significantly different at baseline between the cohorts (p=0.11). *IL1β* gene expression was significantly upregulated in response to LPS in controls (p<0.0001) but not in children with SNI (p=0.08).
Figure 5.11. Expression of genes from the Nucleotide-binding and oligomerization domain (NOD)-like receptor protein 3 (NLRP-3) inflammasome at baseline and following exposure to 1uL lipopolysaccharide (LPS; 10ng/ml) exposure for 1 hour.

Gene expression of NLRP3, Apoptosis-associated speck-like protein containing a CARD (ASC) and Interleukin 1β (IL1β) was quantified by real-time polymerase chain reaction (PCR). Values are expressed as fold changes in gene expression following incubation with LPS (10ng/ml) for 1 hour. Two way ANOVA (mean±SD); Control (Con, n=6); Severe Neurological Impairment (SNI, n=14); Veh, Vehicle; *p≤0.05; ****p≤0.0001.
5.5 Discussion

In this study, we have demonstrated significant differences in the proportions of several innate cells, as well as in the expression of various functional markers, in children with SNI. We have shown a significant reduction in total T cell numbers in children with SNI in comparison to controls. T cells are generated in the thymus and function as part of the adaptive immune response. Most T cells express an \( \alpha \beta \) T cell receptor complex (TCR) although a smaller subset express a \( \gamma \delta \) TCR. They play a crucial role in host defence against viruses and intracellular bacteria as well as regulating the inflammatory response. Therefore, any alteration in T lymphocyte proportions or function may contribute to inflammatory dysregulation or a reduced capacity to respond to infection. Taher et al(497) described an increase in T cell numbers in children with CP compared to controls. This may appear to conflict with our findings, however, Taher et al focussed on children with NE and CP, while we have used the term SNI to define our population. There may be significant differences in aetiology, functional status, age range and co-morbidities between the groups, creating difficulty in directly comparing the two cohorts.

We have also shown a reduction in CD8+ cytotoxic T cells in children with SNI. Cytotoxic T cells express CD8, a heterodimeric co-receptor consisting of a CD8\( \alpha \) and a CD8\( \beta \) chain. These T cells play an important role in host defence against intracellular pathogens, such as viruses and intracellular bacteria, by recognising peptides bound to Major Histocompatibility Complex (MHC) Class 1 found on all nucleated cells. Once an antigen is recognised, CD8+ T cells kill the infected cell through a number of mechanisms including cytokine production, release of cytotoxic granules and Fas/FasL interactions. Our findings differ from the study by Taher et al, who did not find a significant difference in CD8+ cells in children with CP or following NE(497). However, as mentioned previously, the populations in the two studies may not be comparable. Evidence of the clinical consequences of a reduction in cytotoxic T cells may be seen in the immune dysfunction of children with 22q11 deletion syndrome (22q11DS). The phenotype, including immunodeficiency, is highly variable and only a minority present with the most severe immune deficit, Severe Combined Immunodeficiency (SCID). Many more have a milder phenotype where cytotoxic T cell numbers, immunoglobulins and vaccine responses are often low. These children have reduced responses to polysaccharide antigens, e.g. *Pneumococcus*, resulting in a predisposition to recurrent respiratory tract infections(502, 503). It is not yet clear whether the reduced population of CD8+ cells in our study would translate to a similar, clinically important, predisposition.

Proportions of other lymphocyte subsets (including CD4+, \( \gamma \delta 1 \) and \( \gamma \delta 2 \) T cells; NK cells; and B cells) were not significantly different between the control and SNI groups. There was a trend towards reduction in \( \gamma \delta 1 \) T cells which did not reach statistical
significance. CD4+ T helper cells facilitate other aspects of the immune response, through the production of cytokines, on recognition of antigens bound to MHC class 2 molecules on antigen presenting cells. CD4+ T regulatory cells maintain homeostasis and self-tolerance. γδ T lymphocytes are CD4 and CD8 negative and make up a small proportion of lymphocytes. They have a propensity for mucosal and epithelial tissues, pointing to a role in the “first line of defence” in these tissues. Their response to stimulation has similarities to both the adaptive and innate immune responses, sometimes described as “adaptate”(504, 505). The trend towards reduced γδ1 proportions is supported by the study by Taher et al, where a significant reduction in γδ1 cells was seen in CP(497). A trend towards increased NK cells was also noted, a pattern which has previously been reported in neonates with NE but not in school-age children post-NE or with CP(497).

Proportions of neutrophils was not different between the groups. On neutrophils, neither TLR4 nor CD11b showed a significant difference in expression between the cohorts, although, CD11b increased, following LPS exposure, in both controls and in children with SNI. The role of neutrophils in neuro-inflammation and neuro-immune interactions is increasingly recognised (506). Neutrophils form part of the innate immune system and often represent the first line of defence against infection(507). They are activated by products of bacteria and tissue damage and carry out their function through a variety of mechanisms including phagocytosis, degranulation, release of reactive oxygen species, and neutrophil extracellular traps (NETs)(506). Cellular activation is triggered, on recognition of pathogenic stimuli, through transmembrane receptors such as the TLRs which are expressed by neutrophils and monocytes, amongst others(507, 508). CD11b can act as a marker of activation. It is expressed by many cells of the innate immune system including monocytes, granulocytes and NK cells. CD11b is involved in cell adhesion, spreading and migration as well as supporting phagocytosis and chemotaxis(509).

TLR4 expression on neutrophils was not different between the control group and those with SNI, nor did it change significantly with exposure to LPS. This may be in keeping with findings by Sabroe et al, that TLR4 expression on neutrophils is not upregulated in response to LPS stimulation(510), although, in contrast to our study, the study by Sabroe et al was not performed on whole blood so the findings may not be comparable. It is also possible that the patients in this study were hyporesponsive to LPS stimulation. TLR4 is functional at low levels(510) and so significant upregulation on neutrophils may not necessarily be required to increase responsiveness. The expression of TLR4 mRNA, in neutrophils, has been shown to increase following incubation with LPS for 3 hours(511). Thus, the lack of neutrophil TLR4 upregulation in our population may be due to the shorter incubation with LPS and/or differences in how TLR4 expression is regulated at the protein and mRNA levels(510).
Expression of CD66b increased significantly in both children with SNI and controls following exposure to LPS. However, children with SNI exhibited CD66b-hyporesponsiveness compared to controls. CD66b is a glycosylated protein which is expressed by human granulocytes and can serve as a marker of activation in these cells(512) and plays a role in cell adhesion and migration in response to stimulation(513, 514). Significant upregulation of CD66b is seen in sepsis. Increased expression of CD66b is associated with neutrophil aggregation and cell adhesion(515). Cross-linking of the molecule leads to an oxidative burst and IL-8 release(516). Higher CD66b expression is thought to lead to more aggregate formation, more opportunity from cross-linking events and, therefore, increased adhesion activities of neutrophils(515). In adults, Kobold et al described leukocyte activation in sepsis and found that reduced activation of both monocytes and neutrophils at diagnosis correlated with higher mortality(517). In the paediatric setting, Weinshenk et al demonstrated that preterm infants with culture-proven or suspected sepsis respond with increased CD66b on leukocyte cell surfaces, but did not correlate the extent of expression with clinical outcome(518). Due to the lack of such research in children, it is not certain that the aforementioned findings of Kobold et al would translate to the paediatric setting. In our cohort, it is possible that CD66b hyporesponsiveness may translate into increased morbidity and mortality related to infection. Immune dysfunction in sepsis may be particularly relevant in the cohort of children with SNI because the nervous system plays a central role in the inflammatory response to systemic infection(519, 520).

We have demonstrated a reduced proportion of monocytes, at baseline, in children with SNI compared to controls. Monocytes are agranulocytes which function as part of the innate immune system through phagocytosis, antigen presentation and cytokine production. There are 3 distinct subsets of monocytes which are differentiated by their expression of CD14 and CD16: classical monocytes (CD14+/CD16-), intermediate monocytes (CD14+/CD16+) and non-classical monocytes (CD14dim/CD16+). It has been proposed that classical cells, mobilised in response to inflammation, differentiate to non-classical monocytes, with intermediate monocytes acting as a transitional phase(521). Other authors advocate that intermediate monocytes represent a distinct subset, with their own discrete function(522). It is clear that monocytes are essential in early response to tissue injury and in bridging the gap between the innate and adaptive immune systems(522, 523). It is, therefore, possible that there may be a detrimental effect of a relative paucity of monocytes on immune function. In adults with sepsis, Chung et al reported that lower monocyte counts were associated with higher mortality, rate of bacteraemia, mechanical ventilation, vasopressor use and renal replacement therapy(524). A national, population-based prospective study in France (n=818; including 107 maternal-neonatal infection) examined the prognostic factors of listeriosis and found
monocytopenia to be one of the strongest mortality predictors (OR 3.70 [1.82-7.49], p=0.0003)(525). In children with cancer, monocytopenia has been associated with higher rates of documented infection(526). Monocytopenia has also been reported to be associated with higher mortality in Coronavirus Disease 2019 (COVID-19) in adults with type 2 diabetes mellitus(527); in invasive pulmonary Aspergillosis infections complicated by respiratory viral infections in patients with haematologic malignancies(528); and in primary myelodysplastic syndromes(529).

In this study, we have shown that the monocytes of children with SNI had relative hyperresponsiveness of TLR4 expression when exposed to LPS. This relative hyperresponsiveness was seen in the classical, but not in the intermediate or non-classical monocyte subsets. Classical monocytes are considered strongly pro-inflammatory and exhibit superior phagocytosis(522). This may indicate a pro-inflammatory state in children with SNI, as has been previously been suggested by the work of Zareen et al in children with NE and CP(323, 429).

In this study, the expression of NLRP3 and IL1β increased significantly in response to LPS stimulation in controls but not in children with SNI, representing trained immunity, in which prior exposure to LPS causes the cells of the innate immune system to become less responsive to further challenges. This may be advantageous in preventing conditions with an exaggerated inflammatory response, such as the cytokine storm which may be seen in sepsis and is associated with increased mortality(530). On the other hand, there may be a reduction in the innate inflammatory response to infection indicating a relative immunosuppression(430).

The NLRP3 inflammasome is an essential component of the innate immune system. Assembly of the complex leads to release of pro-inflammatory IL1β and IL18 as well as pyroptotic cell death(499). The NLRP3 inflammasome has been implicated in numerous disease states including inflammatory bowel disease(531), neurodegenerative disorders(532) and sepsis(533). It has generated considerable interest as a potential therapeutic target for many disorders in which it has been proposed to play a role(534). In neonates, dysregulation of NLRP3 has been demonstrated in NE and this dysregulation persists in childhood(430). Abnormalities in innate immune signalling have been linked with many neurodevelopmental disorders including autism and schizophrenia(535).

A limitation of this study is the relatively small sample size. However, there is a significant paucity of research in the area of immune and inflammatory dysregulation in children with neurodisability. This study raises immune dysregulation as a potential contributory factor to the increased infection-related morbidity and mortality in SNI. Interpretation of the results is complicated by the heterogeneous nature of the population of children with SNI. However, the same can be said for other neurodevelopmental disorders such as autistic spectrum disorder and cerebral palsy, which are frequently considered
together for research purposes. Considering the rarity of many of the conditions which lead to SNI, a much greater number of participants would be required to be able to factor the aetiology of SNI into our interpretation of the results. In using peripheral blood for this study, we are unable to comment on whether a redistribution of immune cells, for example to the CNS, could be contributing to the relative reduction of T cells and monocytes which we have described here.

Relatively little literature exists on innate and adaptive immune cell proportions and function in children with neurological impairment. Alterations in immune function have the potential to, at least partially, explain the increased burden of infection-related morbidity and mortality in this population. In addition, the cells of the immune system are central to initiating, maintaining and abolishing the inflammatory response. In health, pro- and anti-inflammatory influences are finely balanced and dysfunction can lead to abnormal response to infectious stimuli(536), multi-organ dysfunction(537), auto-immunity, and tertiary neurological damage(538). We have described altered proportions of a number of lymphocyte and granulocyte sub-populations in children with SNI. We have also illustrated alterations in the response of neutrophils and monocytes when exposed to the endotoxin LPS.

Children with CP and those who have had neonatal encephalopathy (NE) are known to have persistent inflammation into school-age(127, 429). It is speculated that this persistent inflammation may lead to tertiary brain damage, in particular if exposed to a second or subsequent “hit”(285). Lymphocytes are likely to play a key role in this inflammatory dysregulation.

We have found significant differences in immune regulation in children with SNI compared to controls. Future prospective longitudinal studies which correlate these differences with health-related outcomes are required. However, there is much interest in the targeting of immune cells and the inflammasome for therapeutic manipulation and these immunomodulatory therapies may be useful in improving health-related outcomes in children with SNI.
Chapter 6 – Multi-Organ Dysfunction in SNI
6.1 Introduction

Children with SNI have significant medical complexity and almost every organ system may be potentially affected (491). This has significant repercussions for the child, their family, and the health service (373, 539). It is known that children with neurological impairment, although few, require many resources to maintain health and quality of life (540). This has implications for planning of healthcare services in the medium and long term, but there are also implications for the child and family who need optimal care for their unique needs.

To date, the extent of multi-organ dysfunction in children with SNI has not been quantified. This may have utility in monitoring health, tracking natural history over time and may assist in discussions regarding prognosis in children with SNI.

First, we describe renal function in children with SNI, including the difficulties with assessing this in clinical practice. We then describe some markers of cardiac function, with consideration given to future directions in measuring subtle cardiac dysfunction in these children. Finally, we quantify the involvement of multiple organ systems in our cohort of children with SNI. We describe a preliminary scoring system which illustrates how a future system for multi-organ dysfunction may appear, and discuss the utility of such a score in the assessment of these children, and in tracing the natural history of their health status over time. A formal process for development and validation of this tool will be required in the future before it can be used in clinical or research practice. Nevertheless, the score we show here may provide a useful starting point from which to begin the process.
6.2 Renal dysfunction in children with SNI

6.2.1 Background

Children with SNI are at increased risk of dysfunction in several organ systems (491). Children with neurodevelopmental disorders are more likely to have issues with the genitourinary tract, which may, in turn, lead to renal dysfunction. Lower urinary tract symptoms and urodynamic abnormalities are greater in those with poorer motor function (31), and bladder dysfunction, including urinary retention, has been linked with upper urinary tract deterioration in children with CP (159). There may be progression of urodynamic abnormalities over time as adults with CP, especially those at GMFCS III-V, are noted to be more likely to have small high-pressure bladders, which increases the risk of developing renal dysfunction (160). Adults with neurodevelopmental disorders are noted to be at higher risk of chronic kidney disease than controls (176).

Urinary tract infections (UTI) are more common in children with CP than the general population (161), and recurrent febrile UTIs are known to be associated with upper urinary tract deterioration and renal scarring (541).

Further concern regarding the development of renal dysfunction in children with SNI is raised by studies which have examined children following neonatal encephalopathy (NE) and have found a significant proportion of infants have renal involvement (173). This is associated with neurological outcome, with evidence that acute kidney injury in the neonatal period is associated with the development of chronic kidney disease in later life (168-170, 172-175).

The diagnosis of renal dysfunction is complicated by difficulties in the use of creatinine in monitoring renal function in children with SNI (542). The production of creatinine is linked to muscle mass (543). Those with reduced mobility and resultant reduction in lean muscle mass will, therefore, have lower creatinine levels at baseline (544). Blood pressure and urinary protein measurements can assist in detection of kidney disease, but neither are specific enough to provide a diagnosis of renal impairment.

Due to the inherent difficulties of using creatinine in this cohort we have included measurement of cystatin C. Cystatin C is a low molecular mass protein which is consistently produced by human cells (543), and is unaffected by muscle mass (542), making it preferable for monitoring renal function in children with SNI.

As a result of the existing evidence suggesting that kidney dysfunction may exist as a comorbidity in children with SNI, we evaluated biochemical markers of renal function and compared them to controls.
6.2.2 Hypothesis

In comparison to a control population of healthy children, children with SNI have renal dysfunction, as measured by Cystatin C, a biochemical marker which is independent of muscle mass.

6.2.3 Aims

To examine whether children with SNI have biochemical markers of renal dysfunction in comparison to a control population of children who do not have any chronic health conditions.

Deliverables:
1. A comparison of urea, sodium, potassium, and creatinine levels in children with SNI versus controls.
2. Cystatin C is a marker of renal function which is independent of muscle mass. We will compare Cystatin C values in children with SNI compared to controls, as a method of detecting subtle renal dysfunction in this cohort.
6.2.4 Results

6.2.4.1 Demographics

All participants were recruited from CHI at Tallaght as described in section 2.4.1. Children from the control group (n=12) were in the age range 1.25 to 15.25 years (mean=8.10±4.45) and were undergoing phlebotomy. None had a chronic medical condition, acute infection, or neurodevelopmental disorder.

Children in the SNI cohort (n=23) were aged between 0.7 and 16.5 (mean±SD; 10.79±4.87) years and attended the complex needs clinic in CHI at Tallaght. Age was not significantly different between the control and SNI groups (p=0.11). All children with a diagnosis of SNI were defined as in chapter 3, i.e., a permanent disorder of the CNS which resulted in motor impairment, cognitive impairment, and medical complexity, where much assistance was required with activities of daily living(428). All children in the SNI group were classed as having Gross Motor Function Classification System (GMFCS) IV-V. Children with a range of neurodevelopmental disorders were recruited, including CP (n=14) of varying aetiology, Rett syndrome (n=4), Wolf-Hirschhorn syndrome (n=2), Genitopatellar syndrome (n=1), Calcium/Calmodulin Dependent Serine Protein Kinase (CASK) mutation (n=1), and Arthrogryposis (n=1). Documented co-morbidities of the genito-urinary tract included: recurrent UTI (n=4), urinary retention (n=2), and renal calculi (n=1).

6.2.4.2 Renal function results

Sodium, potassium, and urea values were similar between the groups with no significant differences seen (p=0.81, p=0.75, and p=0.82 respectively; Table 6.1; Figure 6.1). Creatinine was significantly lower in the children with SNI than in controls (p=0.04 Table 6.1; Figure 6.1). There was no difference seen in Cystatin C in controls compared to children with SNI (p=0.08; Table 6.1; Figure 6.1).
Table 6.1. Markers of renal function in children with severe neurological impairment (SNI) versus controls. Data are displayed as mean±SD unless otherwise stated * represents a p value ≤0.05

<table>
<thead>
<tr>
<th></th>
<th>Control (mean±SD)</th>
<th>SNI (mean±SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium mmol/L</td>
<td>139.20±1.99</td>
<td>138.90±3.44</td>
<td>0.82</td>
</tr>
<tr>
<td>Potassium mmol/L</td>
<td>4.02±0.23</td>
<td>4.06±0.43</td>
<td>0.75</td>
</tr>
<tr>
<td>Urea mmol/L</td>
<td>4.6±1.41</td>
<td>4.49±1.33</td>
<td>0.82</td>
</tr>
<tr>
<td>Creatinine umol/L</td>
<td>33.5 (23.00-43.00)</td>
<td>23.00 (21.00-31.00)</td>
<td>0.04</td>
</tr>
<tr>
<td>(median, 95%CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystatin C pg/ml</td>
<td>659.70 (464.00-811.40)</td>
<td>508.00 (428.30-643.00)</td>
<td>0.08</td>
</tr>
<tr>
<td>(median, 95%CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.1. Markers of renal function in children with SNI versus controls.

Cystatin C was quantified using Enzyme Linked Immunosorbent Assay; Urea, Creatinine and electrolytes were analysed as part of routine clinical care in the Hospital laboratory. Urea, Sodium and potassium were normally distributed and analysed using unpaired student t tests (mean, SD). Creatinine and Cystatin C were not normally distributed and were analysed using Mann Whitney U test (median, 95%CI). Controls (Con; n=12); Severe Neurological Impairment (SNI; n=23). * represents a p value ≤0.05
6.2.5 Discussion

We have demonstrated that children with SNI have significantly lower creatinine levels than controls. This is consistent with the literature (179, 544) in which reduced creatinine is seen in children with neurodisability. Creatinine is derived from the breakdown of creatine, released into the plasma at a relatively constant rate, and is freely filtered by the glomerulus (543). There are issues with the use of creatinine to monitor renal function in the general population, including that 10-40% of creatinine is cleared by tubular secretion, with the result that there may be a significant decline in glomerular filtration rate (GFR) before increases in creatinine are seen (543). In children with neurodisability, this difficulty is compounded by the fact that restricted mobility, with resultant reduction in muscle mass, is associated with a reduction in creatinine at baseline (179, 544), as we have demonstrated. Thus, any decrease in GFR may be concealed by falsely reassuring creatinine levels.

GFR is considered the best measure of kidney function, but accurate measurement is cumbersome and invasive, particularly in children with SNI. Accurate quantification of GFR may be evaluated using inulin clearance over time, which requires intravenous access and timed collection of blood and urine samples (542). The latter may be difficult in children with SNI, who are most often incontinent of urine, and would therefore require bladder catheterisation.

Estimated GFR (eGFR), calculated with a variety of different formulas, is therefore, frequently used in everyday clinical practice. There are inherent difficulties in the use of these formulas for children with SNI. Firstly, most of the formulas, e.g., the “bedside” Schwartz equation (545), rely on measurements of height and/or weight as a method of estimating muscle mass/body surface area which is factored into the calculation. In children with SNI, accurate measurements of height and weight can be difficult due to poor mobility, increased tone, and contractures. Also, even if these variables were accurately measured, body composition is different due to reduced lean muscle mass with a relatively higher adiposity (546, 547). Other eGFR formulas rely on average creatinine values for age and sex (548), which may not be applicable to children with SNI.

Cystatin C levels were not significantly different in controls compared to children with SNI. Cystatin C has the ability to diagnose kidney injury earlier, with more accuracy, and with a better relationship to disease severity (543). It is, therefore, more reassuring that we see no difference between our cohorts. Nevertheless, it is preferable to use eGFR than absolute values of either creatinine or Cystatin C (549), a task which is rendered more difficult by the aforementioned problems with these equations in children with SNI.

The limitations of this study include the small numbers of participants and the lack of information on body composition. The children with SNI also have relatively
heterogeneous diagnoses, however this is similar to the heterogeneity seen in the CP classification and diagnoses(550).

In summary, we have demonstrated significantly lower creatinine levels in children with SNI compared to controls. There was no significant difference in Cystatin C, which may be a more reliable marker of renal function in these children. Larger longitudinal studies, with accurate anthropometric data, and which follow these children into adulthood including blood pressure measurement would be useful to track changes in renal function over time. It may also be useful to develop and validate an eGFR equation for children with SNI or other neurodisabling conditions to allow accurate monitoring of renal function in this population.
6.3 Cardiac dysfunction in children with SNI

6.3.1 Background

Children with SNI have medical complexity (428), with the potential for dysfunction in multiple organ systems, including the cardiovascular system (491).

Children with neurological impairment are at increased risk of mortality at a younger age than the general population (492). Since 1990, mortality for those with the most severe impairments has increasingly shifted from childhood to early adulthood. In adults with CP, cardiovascular disease is one of the most common causes of death (54), and they have a 3-fold increased risk of mortality due to diseases of the circulatory system (124). The reasons for this are likely multi-factorial including reduced mobility, reduced lean-muscle mass, and increased adiposity (551). Adults with CP are also more likely to have "non-traditional" risk factors for cardiovascular disease, including increased carotid artery intima media thickness, and reduced flow-mediated dilation, which occur at a younger age and progress faster than the general population (552).

High-sensitivity Troponin T (hsTroponin T) and N-terminal prohormone b-type natriuretic peptide (NT-proBNP) were measured as markers of cardiac function which are readily available in the clinical setting. The Troponin complex regulates muscle contraction of all striated myocytes. Troponin T is one of the three subunits making up this complex (553), and the cardiac isoform of Troponin T is highly sensitive and specific for detection of damage to cardiomyocytes (554). BNP is produced as a prohormone by the heart in response to stretch, hypoxia, and certain hormonal influences (555). ProBNP is split into active BNP and the remaining NT-proBNP, the latter having a longer half-life and, thus, being considered more useful from a diagnostic perspective (555).

Troponin T has long been used as a marker of acute myocardial damage, such as that seen with myocardial infarction (556). In the paediatric population, Troponin has been shown to correlate well with outcomes following cardiac surgery and may have value in diagnosis of acute cardiac disorders, such as pericarditis or cardiac contusion (557). Troponin has also been shown to be elevated in children with congenital heart defects, including atrial septal defects, ventricular septal defects, and single ventricle hearts, possibly due to volume overload increasing myocardial oxygen demand (557). In other chronic, non-cardiac, conditions in childhood, Troponin has been used to monitor for cardiac complications. In children with chronic kidney disease, elevated Troponin has been shown to correlate with echocardiographic findings of cardiac dysfunction, even before the development of symptoms, and the authors suggest it may be useful for screening purposes (558). In motor neurone disease, Troponin is elevated and increases longitudinally, suggesting it may be useful in tracking disease progression in this population (559).
NT-proBNP is most often used in the evaluation of heart failure, as it is released in response to cardiac myocyte stretch (555). It is used in screening for heart failure amongst children with congenital heart disease (560). Routine measurement of BNP is also recommended in children undergoing peritoneal dialysis to screen for morphological and functional cardiac problems (561). In children with type 1 diabetes mellitus, NT-proBNP is elevated and is associated with subtle cardiac dysfunction (562). In adults with neurocardiac injury (cardiac dysfunction associated with a neurological insult), elevated BNP has been found to be associated with poor outcomes (563).

Older children with CP are known to have reduced heart rate variability (564); children with epilepsy have reduced systolic and diastolic function, even when seizure free (140); and children following traumatic brain injury have alterations in myocardial strain which persist for at least one week after the initial injury (141). Severe neurological injuries have been implicated as a cause of cardiac dysfunction (142). Cardiac dysfunction in children with SNI has not been adequately explored in the literature. We measured high sensitivity hsTroponin T and (NT-proBNP), both markers which relate to cardiac function, and compared them with controls.

6.3.2 Hypothesis
Children with SNI have cardiac dysfunction, as demonstrated by raised levels of hsTroponin T and NT-proBNP in serum, in comparison to a cohort of healthy controls.

6.3.3 Aim
To assess for biochemical markers of cardiac dysfunction in children with SNI versus controls.

Deliverables:
A comparison of hsTroponin T and NT-proBNP levels in serum, as markers of cardiac dysfunction, in children with SNI compared to a healthy control population of children.

6.3.4 Results
6.3.4.1 Demographics
All participants were recruited from CHI at Tallaght. Children from the control group (n=14) were in the age range 1.25 to 15.25 (mean= 8.10±4.45) years and were undergoing phlebotomy. None had a chronic medical condition, acute infection, or neurodevelopmental disorder.

Children in the SNI cohort (n=13) were aged between 0.66 and 16.5 years (10.25±5.45) and attended the complex needs clinic in CHI at Tallaght. Age was not
significantly different between the control and SNI groups (p=0.27). All had SNI as defined in chapter 3, i.e., a permanent disorder of the CNS which resulted in motor impairment, cognitive impairment, and medical complexity, where much assistance was required with activities of daily living(428). All children in the SNI group were classed as having Gross Motor Function Classification System (GMFCS) IV-V. Children with a range of neurodevelopmental disorders were recruited, including CP (n=9) of varying aetiology, Rett syndrome (n=1), Wolf-Hirschhorn syndrome (n=2), and Calcium/Calmodulin Dependent Serine Protein Kinase (CASK) mutation (n=1). Three of the children with SNI were reported to have had a structural abnormality of the heart which did not require intervention.

6.3.4.2 hsTroponin T and NT-proBNP

There was no significant difference in hsTroponin T values in controls versus those with SNI (median 95%CI; 14.00 (14.00-14.00) vs 14.00 (14.00-14.00); p=0.74; Figure 6.2). Similarly, there was no difference in NT-proBNP between the groups (Controls vs SNI; 50.00, 95%CI 50.00-67.00 vs 50.00, 95%CI 50.00-85.00; p=0.51; Figure 6.2).
Figure 6.2. Markers of cardiac function in controls compared to children with SNI.

Mann Whitney U test (median, 95%CI). Controls (Con; n=14), Severe Neurological Impairment (SNI, n=13)
6.3.5 Discussion

We have demonstrated that there is no significant difference in HS Troponin T or NT-BNP in children with SNI compared to controls. At a single time-point, we have shown no difference in Troponin levels between children with SNI and controls. However, longitudinal studies, with larger numbers of participants would be useful to make definitive conclusions regarding Troponin and cardiac dysfunction in SNI.

We found no difference in NT-proBNP levels in children with SNI compared to controls. The usefulness of BNP in screening for cardiac dysfunction in children with neurological impairment is not well described in the literature. Our finding that NT-pro BNP levels are not significantly different between those with SNI and controls, maybe reassuring. However, as with Troponin, our sample size was small and samples were only taken at a single time-point, so, further research is needed to assess the usefulness of BNP in children with SNI over time.

Children and adolescents with CP have a high frequency of cardiometabolic risk factors, particularly in children with lower mobility(565). Children with CP with poorer motor function and those with vitamin D deficiency had elevated plasma triglycerides(565) Female sex and those with a higher BMI (on CP-specific charts) is associated with insulin resistance(565). Adults with CP have high rates of cardiometabolic disease such as atherosclerosis and hypercholesterolaemia(566). It may be that individuals with CP begin accumulating cardiometabolic risk factors in childhood. Work is underway to examine the effectiveness of physical activity programs in children and young adults with CP in reducing these risk factors(567).

Sweetman et al report that in neonatal encephalopathy, infants commonly develop cardiac dysfunction, as demonstrated by rises in Troponin and BNP(568). Troponin T levels are correlated with MRI findings and neurodevelopmental outcomes in these children(146). The authors suggest that follow-up studies are warranted to ensure optimal cardiac function in later life(568), although, to date, no studies have been performed in children examining whether this cardiac dysfunction persists.

We did not assess for echocardiographic signs of cardiac dysfunction in our study. Advances in technology have allowed more detailed analysis of cardiac function, e.g., speckle tracking(569) and tissue doppler(570), meaning it may be possible to detect more subtle cardiac dysfunction in these children. In childhood cancer survivors, these techniques have proven utility in detecting sub-clinical cardiac dysfunction(571). Similarly, in cystic fibrosis and nephropathic cystinosis, speckle tracking and tissue doppler have detected subtle cardiac dysfunction in children(572, 573). This raises the possibility of using these techniques in children with SNI to monitor for signs of sub-clinical cardiac dysfunction. If echocardiogram findings correlate well with Troponin and BNP in children
with SNI, this may assist with screening in areas where echocardiograms are not routinely available.

Detecting subclinical abnormalities may allow for early intervention to prevent future cardiac events. It is clear that those with neurological impairment have increased cardiac risk factors, including reduced mobility, higher adiposity, and increased CIMT. This translates to higher mortality from cardiac causes, as demonstrated in CP. Therefore, close monitoring of weight and encouragement of as much mobility as possible may be beneficial.
6.4 Multi-organ dysfunction in children with SNI

6.4.1 Background

Children with SNI are likely to have dysfunction in multiple organ systems, as evidenced by that seen in children with CP, where almost every system has the potential to be affected, especially in those children with more severe motor impairment(491). Although children with complex medical needs make up a small proportion of the population, they require a significant number of resources. Also, children with special healthcare needs (CSHCN) with neurological disease have more unmet needs than those without(2). CSHCN with more than two conditions require more visits to healthcare providers, require more services and report more unmet needs, even after adjustment for demographic factors and severity of functional limitation(2). Children with neurological impairments are at increased risk of premature mortality(492). For these reasons, it is important to quantify the extent of multi-system involvement in these children. This may assist with aspects of clinical care, healthcare resource planning, and research.

Several multi-organ dysfunction (MOD) scoring systems have been developed and validated. The Pediatric Logistic Organ Dysfunction (PELOD) score(574), Paediatric Sequential Organ Failure Assessment (pSOFA)(575), and Neonatal Multiple Organ Dysfunction Score (NEOMOD)(576) are common scoring systems to predict morbidity and mortality in the acute setting in critically ill neonates and children.

Horridge et al have developed a Disabilities Terminology Set and have used that information to create a Disabilities Complexity Scale(577). This scale uses “number of conditions, technology dependencies, family reported issues and requirement for round-the-clock-care” as a measure of complexity(577). They have demonstrated that use of this scale and data collected have been beneficial in identifying the needs of these patients and in strengthening the cases for expansion of services to meet these needs(578).

Although the Disabilities Complexity Scale has some similarities, its focus is on needs. To our knowledge, no scoring system exists with a focus on quantifying multi-organ dysfunction. A score such as this may assist in tracking natural history and predicting long-term outcome in children with neurological impairment. We propose a preliminary Multi-Organ Dysfunction (MOD) score for children with SNI, based on the clinical data we collected for SERENITY study, clinical experience, and expert opinion.

A MOD scoring system may have several applications in the clinical and research setting. It may allow for earlier recognition of organ dysfunction, in the same way that other scoring systems in paediatrics allow for the early detection of acute physiological deterioration(579), or malnutrition in hospitalised children(580). It may allow for regular screening of children in the outpatient setting and allow objective assessment of the child’s health over time. A MOD score for children with SNI could also be used to assess the degree of multi-organ involvement in their condition which could help with predictions.
about future outcomes and help to guide conversations with families about goals of care and healthcare needs. Finally, scoring systems can be used in the research setting as an objective measure of health status and outcome measurement.

6.4.2 Research Question
In a cohort of children with SNI, what is the extent of multi-organ dysfunction?

6.4.3 Aims
To quantify and describe multi-organ dysfunction in children with SNI.

Deliverables:
1. A description and quantification of the medical problems seen in each organ system in a cohort of children with SNI, attending a complex needs clinic in a tertiary children’s hospital.
2. Draft a preliminary tool for assessment of multi-organ dysfunction in children with SNI, which can be carried forward to inform a formal process by which a validated and reliable tool can be developed.

6.4.4 Results
6.4.4.1 Demographic information
Twenty-eight children with SNI were recruited from the complex needs clinic in CHI at Tallaght, all of whom met the consensus-based definition of SNI(428). Sixteen of the children were male and the average of the cohort was 8.46 years at recruitment. Most of the children had CP of varying aetiology, while a smaller proportion had rare genetic disorders (Table 6.3). One child did not yet have a formal diagnosis. Most were classified as GMFCS IV-V, although 2 children had not yet received a classification due to their age (Table 6.2).

6.4.4.2 Multi-organ involvement
Clinical information was gathered as outlined in chapter 2. Children with SNI had dysfunction, which we have defined as symptoms or signs requiring ongoing medical attention, in a median of 4 organ systems (95%CI, 3-5; Table 6.3). The participants were prescribed an average of 6.67±3.69 regular medications.

Neurological
In the neurological system, documented bulbar dysfunction(581), epilepsy and spasticity were the most frequent issues encountered by these children. The frequency of bulbar dysfunction was reflected in the high proportion of children who were unable to feed orally. Eight children with epilepsy had more than one seizure type and the average
number of anti-epileptic drugs prescribed to children with epilepsy was 2.44. Spasticity and/or dystonia affected 17 and 8 children respectively, with an average of 2.31 medications prescribed which counter these symptoms. Visual and hearing impairment were prominent issues, affecting 14 and 5 children respectively.

**Gastrointestinal**

The gastrointestinal tract was the second most commonly involved system after the neurological system, with 25 children having at least one problem requiring medical attention. Constipation (n=24) and gastroesophageal reflux (n=21) were the most common symptoms. Visceral hyperalgesia was described in 6 of the children.

**Cardiac & Respiratory**

Relatively few children had known structural cardiac defects, and none required intervention. Nine of the children required antibiotic prophylaxis for recurrent respiratory tract infections. Ten had episodes of apnoea, whether obstructive (n=8) or central (n=2), for which they were prescribed non-invasive ventilation. Two children were unable to tolerate non-invasive ventilation although it had been prescribed.

**Endocrine & musculoskeletal**

Two children had abnormal pubertal onset and two of the female participants had dysmenorrhoea. Vitamin D and Calcium supplementation was frequently prescribed for bone health. Five of the children had had at least one fracture, while 2 children had had more than one in their lifetime. Scoliosis and hip subluxation were significant issues recorded in 15 and 14 cases respectively. In terms of Musculo-skeletal interventions, 8 had required orthopaedic surgery and 9 had undergone treatment with Botulinum toxin (Botox).

**Genitourinary**

With regards to the genitourinary system, four of the children had recurrent urinary tract infections, 2 had urinary retention, and 1 had been diagnosed with renal calculi. Three children had, or were being treated for, anaemia.
Table 6.2. Demographic information for participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (total)</td>
<td>28</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>8.46</td>
</tr>
<tr>
<td>Diagnosis</td>
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</tr>
<tr>
<td>- Cerebral Palsy</td>
<td>18</td>
</tr>
<tr>
<td>- Rett syndrome</td>
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</tr>
<tr>
<td>- Wolf Hirschhorn syndrome</td>
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<td>- Genitopatellar syndrome</td>
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<tr>
<td>- CASK mutation</td>
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<td>- Arthrogryposis</td>
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</tr>
<tr>
<td>- Undiagnosed</td>
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<td>CP type</td>
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</tr>
<tr>
<td>- Spastic bilateral</td>
<td>9</td>
</tr>
<tr>
<td>- Dystonic bilateral</td>
<td>7</td>
</tr>
<tr>
<td>- Mixed bilateral</td>
<td>2</td>
</tr>
<tr>
<td>GMFCS (or equivalent in non-CP SNI)</td>
<td></td>
</tr>
<tr>
<td>- IV</td>
<td>3</td>
</tr>
<tr>
<td>- V</td>
<td>23</td>
</tr>
<tr>
<td>- As yet unclassified</td>
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<td>System</td>
<td>Variable</td>
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<td>------------------------</td>
<td>----------------------------------------------------</td>
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<td>Epilepsy</td>
</tr>
<tr>
<td></td>
<td>- &gt;1 seizure type</td>
</tr>
<tr>
<td></td>
<td>Average number of AEDs</td>
</tr>
<tr>
<td></td>
<td>Dystonia</td>
</tr>
<tr>
<td></td>
<td>Dysautonomia</td>
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<tr>
<td></td>
<td>Spasticity</td>
</tr>
<tr>
<td></td>
<td>Average number of dystonia/spasticity meds</td>
</tr>
<tr>
<td></td>
<td>Bulbar dysfunction</td>
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<tr>
<td></td>
<td>Visual impairment</td>
</tr>
<tr>
<td></td>
<td>- CVI</td>
</tr>
<tr>
<td></td>
<td>- Classification unknown</td>
</tr>
<tr>
<td></td>
<td>Sensorineural hearing impairment</td>
</tr>
<tr>
<td>Cardiac</td>
<td>Structural cardiac defect</td>
</tr>
<tr>
<td></td>
<td>- requiring intervention</td>
</tr>
<tr>
<td></td>
<td>- not requiring intervention</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Recurrent RTIs requiring prophylaxis</td>
</tr>
<tr>
<td></td>
<td>Apnoeas</td>
</tr>
<tr>
<td></td>
<td>- Obstructive</td>
</tr>
<tr>
<td></td>
<td>- Central</td>
</tr>
<tr>
<td></td>
<td>Respiratory support</td>
</tr>
<tr>
<td></td>
<td>- CPAP</td>
</tr>
<tr>
<td></td>
<td>- BiPAP</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Enteral feeding route</td>
</tr>
<tr>
<td></td>
<td>- Oral</td>
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<tr>
<td></td>
<td>- Nasogastric</td>
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<tr>
<td></td>
<td>- PEG</td>
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<tr>
<td></td>
<td>- PEJ</td>
</tr>
<tr>
<td></td>
<td>Visceral hyperalgesia</td>
</tr>
<tr>
<td></td>
<td>Gastroesophageal reflux</td>
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<tr>
<td>Section</td>
<td>Condition</td>
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<td>---------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average number of GI medications</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Vitamin D supplementation</td>
</tr>
<tr>
<td></td>
<td>Calcium supplementation</td>
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<tr>
<td></td>
<td>Abnormal pubertal onset</td>
</tr>
<tr>
<td></td>
<td>Dysmenorrhoea</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Scoliosis</td>
</tr>
<tr>
<td></td>
<td>Hip subluxation</td>
</tr>
<tr>
<td></td>
<td>Orthopaedic surgery</td>
</tr>
<tr>
<td></td>
<td>Botox</td>
</tr>
<tr>
<td></td>
<td>Fractures</td>
</tr>
<tr>
<td></td>
<td>- Single fracture</td>
</tr>
<tr>
<td></td>
<td>- &gt;1 fracture</td>
</tr>
<tr>
<td>Haematological</td>
<td>Anaemia</td>
</tr>
<tr>
<td>Renal</td>
<td>Renal calculi</td>
</tr>
<tr>
<td></td>
<td>Recurrent UTI</td>
</tr>
<tr>
<td></td>
<td>Urinary retention</td>
</tr>
</tbody>
</table>
6.4.4.3 *Multi-organ dysfunction scoring tool*

A preliminary multiorgan scoring tool was created by assigning a score to abnormalities in each organ system. A formal development and validation process will need to be followed but a future iteration of this scoring tool may be useful in the assessment of children with SNI. It comprises of 21 items, grouped into 8 systems, with a maximum score of 25 (Figure 6.3).

The following systems were included in the scoring system: neurological, cardiovascular, gastrointestinal, haematological, respiratory, musculoskeletal, renal, endocrine and bone health. Each system is further subdivided into individual or clusters of symptoms and signs, with each item being assigned a score. A single point was assigned to each item if present, unless otherwise specified, with 2 points being assigned in certain situations as follows: *Neurological system:* Epilepsy, drug-resistant epilepsy (2 points), bulbar dysfunction, spasticity, dystonia, dysautonomia; *Cardiovascular system:* a structural or functional issue requiring treatment, whether medical, surgical, or both; *Gastrointestinal system:* PEG/NG feeding, PEJ/NJ feeding (2 points), constipation, gastroesophageal reflux, visceral hyperalgesia; *Haematological system:* anaemia, thrombocytopaenia (as defined by normative values); *Respiratory system:* recurrent respiratory tract infections, nocturnal apnoeic episodes, daytime symptoms (not occurring during sleep; 2 points), requirement for respiratory support; *Musculoskeletal system:* scoliosis, hip subluxation; *Genitourinary system:* recurrent UTI, urinary retention, renal calculi; *Endocrine & bone health:* abnormal pubertal onset (whether precocious or delayed), fractures. Drug resistant epilepsy, jejunal feeding and apnoeic episodes which occur outside of sleep were all assigned 2 points as they represent more complex disease.
### SNI MODS scoring tool

**Neurological**
- Epilepsy
  - No: 0
  - Not drug resistant: 1
  - Drug-resistant: 2
- Bulbar Dysfunction
  - No: 0
  - Yes: 1
- Spasticity
  - No: 0
  - Present: 1
- Dystonia
  - No: 0
  - Yes: 1
- Dysautonomia
  - No: 0
  - Yes: 1

Subtotal: □/6

**Respiratory**
- Recurrent respiratory infections
  - No: 0
  - Yes: 1
- Apnoeic episodes
  - No: 0
  - Nocturnal only: 1
  - Daytime symptoms: 2
- Respiratory support
  - No: 0
  - Yes: 1

Subtotal: □/4

**Musculoskeletal**
- Scoliosis
  - No: 0
  - Yes: 1
- Hip subluxation
  - No: 0
  - Yes: 1

Subtotal: □/2

**Cardiovascular**
- Structural or functional requiring treatment
  - No: 0
  - Yes: 1

Subtotal: □/1

**Gastrointestinal**
- Feeding route
  - Oral: 0
  - PEG/NG: 1
  - PEJ/NJ: 2
- Constipation
  - No: 0
  - Yes: 1
- Gastroesophageal reflux
  - No: 0
  - Yes: 1
- Visceral hyperalgesia
  - No: 0
  - Yes: 1

Subtotal: □/5

**Haematological**
- Anaemia
  - No: 0
  - Yes: 1
- Thrombocytopaenia
  - No: 0
  - Yes: 1

Subtotal: □/1

**Renal**
- Recurrent UTI
  - No: 0
  - Yes: 1
- Urinary retention
  - No: 0
  - Yes: 1
- Renal calculi
  - No: 0
  - Yes: 1

Subtotal: □/3

**Endocrine & bone health**
- Abnormal pubertal onset
  - No: 0
  - Yes: 1
- Fractures
  - No: 0
  - Yes: 1

Subtotal: □/3

**Total Score**: □/25

---

**Figure 6.3. Illustration of how a SNI multi-organ dysfunction scoring tool may be structured.**
6.4.5 Discussion

We have demonstrated that children with SNI have significant medical comorbidities, with dysfunction requiring medical intervention seen in a median of 4 organ systems. There was a significant amount of polypharmacy with children being prescribed an average of approximately 7 regular medications. Neurological issues were the most prominent amongst our cohort. The gastrointestinal, respiratory and musculoskeletal systems were the next most frequently involved, similar to Hollung et al who found disorders of these 3 systems to be the most prevalent in children with CP, although, in contrast, they found disorders of the musculoskeletal system to be more prevalent than those of the gastrointestinal system (587). We propose development of a MOD scoring tool for children with SNI, a preliminary illustration of which we have described here. This may be beneficial for clinical, research and health resource planning purposes, as has been seen with the Disabilities Complexity Scale developed by Horridge et al (577, 578).

The extent of multi-organ dysfunction in children with SNI, which we have demonstrated here, has important implications for the health service. Burns et al demonstrated that hospitalisations of children with complex chronic conditions (CCC) increased significantly over a ten year period (588). This was especially true for children with more than one CCC, where hospitalisations doubled over the same period (588). Of particular relevance to our group was the finding that hospitalisations of children with CP alone decreased, while hospitalisations of children with CP and at least one other CCC increased by 10% every 3 years (588). Children with multiple CCCs have higher mortality rates, higher readmission rates, and higher healthcare costs (539). Children with neurological impairment are the category of Children with Medical Complexity (CMC) most likely to require medical technology assistance and have the highest 2 year mortality (539).

Polypharmacy is often used to describe the regular use of 5 or more medications but this definition relates to the adult population, particularly older adults. Based on an extensive literature review, Bakaki et al have recommended that paediatric polypharmacy be defined as "the prescription or consumption of two or more distinct medications for at least one day" (589). Interestingly, young adults with CP and other neurodevelopmental disabilities have been found to have a similar prevalence of polypharmacy as that seen in the elderly population (590). A large retrospective cohort study (n=9238) in adults with CP showed that polypharmacy is a risk factor for severe chronic kidney disease, liver disease and mortality (591). In the paediatric population, outside of epilepsy and psychiatric disorders, there is a paucity of literature regarding polypharmacy in children (592). Feinstein et al have combined parent-reported symptoms with medication use in children with SNI and tested whether a higher overall symptom score was associated with higher medication use (593). In keeping with our findings, they found that children with SNI experience a significant symptom burden and significant polypharmacy; 76% of the
participants enrolled in their study were prescribed more than 10 regular medications(593). This is in line with our report that children with SNI averaged approximately 7 regular medications. It is also of note that they sought to use a standardised score to assist with accurate data collection and interpretation. However, the tool used by Feinstein et al assessed parent-reported symptoms(593), whereas the tool we have proposed in this chapter focusses on organ dysfunction. As many symptoms may be attributable to the same underlying cause, these tools are not strictly analogous. Interestingly, in the Feinstein study, they reported that over 70% of children with SNI had 3 or more co-existing CCCs(593), which concurs with our report that these children have dysfunction in a median of 4 organ systems.

A review of multi-organ dysfunction scoring tools in the acute setting, showed that they were useful for predicting mortality but that performance varied widely(594). The authors identified the need for unified, organ dysfunction criteria to improve consistency(594). Although a MOD score for SNI may be more useful in the non-acute setting, it will be similarly important to have well defined criteria to define “dysfunction” in each organ system. The authors also note the utility of the Delphi process in creating such criteria and in the development of scoring tools(594). This may be a useful technique to develop and refine a SNI MOD score, which will then need to be validated in large population based studies.

The SNI MOD scoring tool may prove useful in the clinical setting to objectively assess the extent of MOD in a child with SNI, to track symptom development over time, to assist with early detection of organ dysfunction and declining health. With further validation, it may be useful for research purposes or discussions with families regarding prognosis. Integration of the tool with a core outcome set would enhance the usefulness and validity of the tool.

Limitations of this study include the small numbers of participants and the retrospective nature of our data collection. It is worth noting that Ireland does not have a well-developed hip screening program for children with CP, such as is seen in some other countries(595). There may therefore be a higher rate of hip subluxation seen in Ireland, however, a national survey of children with CP would be required to compare the rates of hip subluxation here with those of a country such as Sweden, where a hip surveillance program is in operation(595). Future work will focus on developing, refining and validating the SNI MOD scoring system. Also, the definitions of certain terms have not yet been agreed internationally. For example, dysautonomia has several synonyms/related terms including autonomic storm, sympathetic storm, paroxysmal sympathetic hyperactivity etc(583). We have tried to remedy this situation by ensuring that we have referenced a definition of each these terms as they have been interpreted for the SNI MOD score. In refining the score in the future, consensus may be sought around the most widely
accepted terminology for a particular issue, with consideration given to new consensus-based definitions as/if they arise.

In summary, children with SNI have significant dysfunction in multiple organ systems, with particular issues noted, outside of the CNS, in the gastrointestinal, musculoskeletal and respiratory systems. Polypharmacy occurs very frequently in this group of children, and is under-researched. It is certain that the complexity of these children’s needs represent a significant source of distress for them and their families, and contribute significantly to health resource use. A scoring system to quantify multi-organ involvement in children with SNI may be useful in the clinical and research settings.

6.5 Conclusion

In this chapter we have reported on multi-organ dysfunction in children with SNI, with a particular focus on the renal and cardiovascular systems. We then quantified the extent of their medical needs and proposed a preliminary scoring system which may be of benefit in future work. We have shown that there are no significant differences in biochemical markers of renal and cardiac function. A reliable method of calculating eGFR would be of value for children with neurodisability. With regards to the cardiovascular system, future work focussing on echocardiographic measurements may allow us to detect sub-clinical cardiac dysfunction in this population. Finally, following further refinement and validation, the SNI MOD score may prove a valuable tool for early detection of declining health, prediction of health-related outcomes, and identification of needs.
Chapter 7 – Wellbeing of Families who Care for a Child with Severe Neurological Impairment
7.1 Introduction

Children with Severe Neurological Impairment (SNI) have complex medical needs and require a significant amount of assistance with activities of daily living (428). Caring for children with significant neurological disorders can have a significant impact on the family (596, 597) with disruption to the family routine, breakdown of family relationships and the introduction of healthcare professionals into the family home (598-600).

Much of the research on family wellbeing focuses on parental and, in particular, maternal quality of life in a variety of childhood chronic conditions, including: cerebral palsy (601); atopic dermatitis (602); chronic pain (603); tic disorders (604); osteogenesis imperfecta (605); and patients with tracheostomies (606). There is a growing body of research examining the experiences of fathers in caring for a child with a long-term illness but their responses are not yet widely understood (607). However, it appears that the experience of fathers differs from that of mothers (596), highlighting that, even within the same family, the experiences of family members can be variable.

One of the most important relationships within the family is that between siblings. Siblings play a central role in family dynamics and are important in providing socialisation for one another (608, 609). They are considered one of the most important influences in shaping social and cognitive development (610). There is a paucity of research on the wellbeing of healthy siblings of children with chronic health conditions. Many of the studies which have been performed have pointed towards a significant impact on the well sibling. Disruptions in the family home resulting from caring for a child with SNI, including frequent hospitalisations, complexities of the child’s condition, and the presence of medical devices in the home, are reported as having a serious effect on the siblings’ emotional, psychological and social wellbeing (599, 611, 612). In contrast, a large longitudinal study in Australia (n=9154) showed minimal changes in some, but not all, indicators of wellbeing in siblings of children with disabilities (344).

Variations between these sibling studies may be due to differences in study design and the measures of wellbeing which are used. It is also important to consider different social and cultural influences internationally. The supports available to children and their families in one jurisdiction may not necessarily be available in another, thus influencing the impact on the family. Also, there is significant variation in the health conditions which have been studied and it cannot be presumed that wellbeing of family members is uniform across the spectrum.

In this chapter, we initially examine family impact using the Pediatric Quality of Life Inventory (PedsQL™) Family Impact Module (FIM); sibling quality of life (QoL) using age appropriate PedsQL™ questionnaires; and the quality of life of the child with SNI using the Caregiver Priorities and Child Health Index of Life with Disabilities (CPCHILD™). In the latter part of the chapter, influenced by input from the families participating in the study,
we describe the findings of a focus group which we performed with teenage siblings of children with SNI. The experiences of family members who live with a child with SNI are important to understand as comprehensively as possible. By deepening our understanding, we hope to provide insights which may help professionals to support parents and siblings in maintaining their own health, wellbeing and development.

7.2 Hypothesis

The families of children with Severe Neurological Impairment (SNI) report poorer quality of life and a significant family impact compared to families of control children who do not have a chronic illness or neurodevelopmental disability.

7.3 Aim

To examine if there is a perceived negative impact on quality of life or family functioning for parents or siblings who care for a child with SNI.

Deliverables:

1. A validated and reliable questionnaire tool, the PedsQL™ Family Impact Module, will be used to measure parental quality of life and family impact in parents of children with SNI, and compared with the responses of parents of healthy controls.

2. A validated, age and developmentally appropriate questionnaire tool, the PedsQL™ Generic Core Scales 4.0, will be used to compare quality of life in siblings of children with SNI and healthy controls.

3. A focus group with teenage siblings of children with SNI will be conducted and analysed by the Interpretive Phenomenological Analysis technique, to provide a more nuanced view of the day-to-day realities of living with a brother or sister who has SNI.
7.4 Results

7.4.1 Family Impact including Parental Quality of Life

7.4.1.1 Demographics

All participants were recruited through Children’s Health Ireland (CHI) at Tallaght. Parents in the control group (n=11) were recruited in the outpatient department when their child attended for routine phlebotomy. None of their children had a chronic illness.

Parents of children with SNI (n=21) were recruited through the complex needs clinic in CHI at Tallaght when their child attended for a routine outpatient appointment. The children with SNI had a range of diagnoses which led to significant motor impairment (GMFCS IV-V), cognitive impairment and medical complexity as a result of a permanent disorder of the central nervous system (428).

7.4.1.2 Parental Health Related Quality of Life

All parents completed the PedsQL™ FIM, version 2.0. This is a 36-item questionnaire which encompasses parental HRQoL, communication, worry and family impact. Parental HRQoL was derived from scores in Physical Functioning, Emotional Functioning, Social Functioning and Cognitive Functioning as per the associated instructions. Each item is scored on a 0-100 scale, with higher scores indicating better QoL.

All data, except for that in the Communication, Worry and Family Relationships domains, were normally distributed as confirmed with the Shapiro-Wilk test. Parents of children with SNI had significantly lower scores than the control parents in the Physical (p=0.006) and Social Functioning (p=0.02) domains (Table 7.1 & Figure 7.1). There was no significant difference in the Emotional (p=0.0525) or Cognitive Functioning (p=0.6576) domains (Table 7.1 & Figure 7.1). There was a significantly lower overall parental HRQoL in the parents of children with SNI compared to the control groups (p=0.0334).

7.4.1.3 Communication and Worry

The Communication subscale asks for parents to rate problems with communicating with others or with healthcare professionals and also whether they believe that others understand their family’s situation. Parents of children with SNI were significantly more likely to report problems with communication than controls (p=0.0005)(Table 7.1 & Figure 7.2).

The Worry subscale relates to worries regarding their child’s treatment, side-effects, reactions of others to their child’s condition, the effect on other members of the family and their child’s future. Parents of children with SNI were significantly more worried than the controls, with a lower median score (p=0.0028)(Table 7.1 & Figure 7.2).
7.4.1.4 Summary scores

The Family Functioning Summary Score (FFSS) is derived from the Daily Activities and Family Relationships subscales. Parents caring for a child with SNI had lower both lower Daily Activity scores (p=0.0002) and lower Family Relationship scores (p=0.014) (Table 7.1 & Figure 7.3).

The FFSS was significantly lower in those families caring for a child with SNI (p=0.0025). The same was true for the Total score (p=0.0029) which takes parental HRQoL, communication, worry and family functioning into account (Table 7.1 & Figure 7.4).
Table 7.1. Pediatric Quality of Life Inventory (PedsQL) Family Impact Module (FIM). Control (n=11); Severe Neurological Impairment (SNI; n=21). Scores are reported as mean (SD) unless otherwise stated. Normally distributed data were analysed using unpaired t-tests, while non-normally distributed data were analysed using Mann-Whitney U tests. P values which have reached a level of significance $\leq0.05$ are highlighted in bold.

<table>
<thead>
<tr>
<th></th>
<th>Control Mean (SD)</th>
<th>SNI Mean (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parental HRQoL</strong></td>
<td></td>
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</tr>
<tr>
<td>- Physical functioning</td>
<td>71.59 (23.92)</td>
<td>46.23 (22.63)</td>
<td>0.006</td>
</tr>
<tr>
<td>- Emotional functioning</td>
<td>62.27 (25.04)</td>
<td>44.76 (22.39)</td>
<td>0.0525</td>
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<tr>
<td>- Social functioning</td>
<td>65.34 (26.42)</td>
<td>41.68 (26.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>- Cognitive functioning</td>
<td>63.18 (27.14)</td>
<td>58.81 (25.78)</td>
<td>0.6576</td>
</tr>
<tr>
<td>- Subscale total</td>
<td>65.63 (22.91)</td>
<td>48.1 (20.19)</td>
<td>0.0334</td>
</tr>
<tr>
<td><strong>Communication; median (95% CI)</strong></td>
<td>91.67 (41.67-100.00)</td>
<td>50 (33.33-58.33)</td>
<td>0.0005</td>
</tr>
<tr>
<td><strong>Worry; median (95% CI)</strong></td>
<td>90 (30.00-100.00)</td>
<td>40 (25.00-50.00)</td>
<td>0.0028</td>
</tr>
<tr>
<td><strong>Family Functioning</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>- Daily activities</td>
<td>67.42 (21.23)</td>
<td>30.16±25.34</td>
<td>0.0002</td>
</tr>
<tr>
<td>- Family relationships; median (95%CI)</td>
<td>75.00 (50.00-100.00)</td>
<td>45.00 (35.00-70.00)</td>
<td>0.014</td>
</tr>
<tr>
<td>- FFSS</td>
<td>71.99 (23.58)</td>
<td>43.3 (23.25)</td>
<td>0.0025</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>69.69 (22.61)</td>
<td>45.24 (18.93)</td>
<td>0.0029</td>
</tr>
</tbody>
</table>
Parents of children with SNI report significantly poorer physical and emotional functioning than controls.

The sub-scores which contribute to Parental Health Related Quality of Life in the PedsQL Family Impact Module are expressed as transformed scores (range 0-100) where lower scores represent poorer functioning. Unpaired t-test (mean, SD). Control (Con, n=11); Severe Neurological Impairment (SNI, n= 21); *p£0.05; **p£0.01.

Figure 7.1. Parents of children with SNI report significantly poorer physical and emotional functioning than controls.
Parents of children with SNI report significantly poorer scores in the Communication and Worry sub-scales of the PedsQL Family Impact Module. The Communication and Worry sub-scores contribute to the total PedsQL Family Impact Module score and are expressed as transformed scores (range 0-100) where lower scores represent poorer functioning. Mann-Whitney U test (median, 95%CI). Control (Con, n=11); Severe Neurological Impairment (SNI, n=21); **p≤0.01; ***p≤0.001.
Figure 7.3. Parents of children with SNI report significantly poorer scores than controls in the Daily Activity and Family Relationships sub-scales.

The Daily Activity and Family Relationships scores contribute to the Family Functioning Summary Score and the total PedsQL Family Impact Module. They are expressed as transformed scores (range 0-100) where lower scores represent poorer functioning. Daily activities scores were normally distributed and analysed using an unpaired t-test (mean, SD). Family Relationships scores were not normally distributed and analysed with a Mann-Whitney U test (median, 95%CI). Control (Con, n=11); Severe Neurological Impairment (SNI, n= 21); *p≤0.05; ***p≤0.001.
Parents of children with SNI have significantly poorer Family Functioning Summary Scores (FFSS) and Total scores than controls. The Daily Activity and Family Relationships scores contribute to the Family Functioning Summary Score. Parental Health Related Quality of Life, Communication, Worry and Family Functioning all contribute to the Total PedsQL Family Impact Module score. They are expressed as transformed scores (range 0-100) where lower scores represent poorer functioning. Unpaired t-test (mean, SD). Control (Con, n=11); Severe Neurological Impairment (SNI, n=21); **p<0.01.
7.4.2 Quality of life of siblings of children with SNI

7.4.2.1 Demographics

Siblings of children with SNI (n=17) were recruited through the complex needs clinic in CHI at Tallaght. The parents of the children with SNI brought home age-appropriate PedsQL™ questionnaires for all siblings aged between 2 and 18 years who lived in the same household. The questionnaires were self-completed by the siblings, if appropriate, and returned by post. For siblings aged 2-4 years, a parent-report form was used. Eighteen siblings of children with SNI returned the PedsQL™ questionnaire.

Healthy children attending for routine phlebotomy (n=9) were asked to complete the same questionnaires as those in the SNI cohort. None of the children or their siblings had a chronic illness or disability. There was no significant differences in the age categories of the SNI and control groups (4.5±2.38 vs 2.25±2.06, p=0.078).

7.4.2.2 Sibling quality of life

There were no significant differences between siblings in the SNI group and controls in Physical (p=0.9293), Emotional (p=0.4227) or Social Functioning (p=0.4258). However, there was a significant difference between groups in the School Functioning subscale with the SNI group having significantly higher scores in this domain (p=0.0389; Table 7.2 & Figure 7.5).

The Psychosocial Summary Score is derived from the Emotional, Social and School Functioning domains. No significant difference was seen between the groups in this score (p=0.1743). The total score combines the scores of the physical and psychosocial elements of the questionnaire. Again, no significant difference was seen here between the groups (p=0.3169; Table 7.2 & Figure 7.6).
Table 7.2. Pediatric Quality of Life Inventory (PedsQL) scores for controls (n=9) and siblings of children with Severe Neurological Impairment (SNI; n=17). Scores are reported as mean (SD) unless otherwise stated. Normally distributed data were analysed using unpaired t-tests, while non-normally distributed data were analysed using Mann-Whitney U tests. P values which have reached a level of significance ≤0.05 are highlighted in bold.

<table>
<thead>
<tr>
<th></th>
<th>Control Mean (SD)</th>
<th>SNI Mean (SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical; median (95% CI)</strong></td>
<td>81.25 (60.00-93.75)</td>
<td>82.82 (65.16-89.84)</td>
<td>0.9293</td>
</tr>
<tr>
<td><strong>Emotional</strong></td>
<td>62.92 (22.41)</td>
<td>69.79 (19.78)</td>
<td>0.4227</td>
</tr>
<tr>
<td><strong>Social</strong></td>
<td>74.44 (16.85)</td>
<td>79.70 (15.42)</td>
<td>0.4258</td>
</tr>
<tr>
<td><strong>School; median (95% CI)</strong></td>
<td>65.00 (45.75-72.03)</td>
<td>75.00 (60.45-87.98)</td>
<td><strong>0.0389</strong></td>
</tr>
<tr>
<td><strong>Psychosocial summary score</strong></td>
<td>65.32 (17.57)</td>
<td>74.35 (14.93)</td>
<td>0.1743</td>
</tr>
<tr>
<td><strong>Total score</strong></td>
<td>68.18 (18.37)</td>
<td>75.31 (16.48)</td>
<td>0.3169</td>
</tr>
</tbody>
</table>
Figure 7.5. Siblings of children with Severe Neurological Impairment (SNI) do not report significantly poorer functioning in Physical, Emotional or Social functioning but have higher School functioning scores than controls.

The Emotional, Social and School Functioning scores all contribute to the PedsQL Psychosocial summary score. All four sub-scores contribute to the Total Peds QL score. They are expressed as transformed scores (range 0-100) where lower scores represent poorer functioning. Data from the Emotional and Social functioning domains were normally distributed and analysed using Unpaired t-tests (mean, SD). Data from Physical and School functioning domains were analysed using Mann-Whitney U tests (median 95%CI). Control (Con, n=9); Severe Neurological Impairment (SNI, n=17); *p≤0.05.
Figure 7.6. Siblings of children with Severe Neurological Impairment (SNI) do not report significantly poorer Psychosocial Health and do not have significantly poorer Total PedsQL scores than controls.

The Emotional, Social and School Functioning sub-scores all contribute to the PedsQL Psychosocial summary score. Physical, Emotional, Social and School Functioning sub-scores contribute to the Total Peds QL score. They are expressed as transformed scores (range 0-100) where lower scores represent poorer functioning. Unpaired t-tests (mean, SD). Control (Con, n=9); Severe Neurological Impairment (SNI, n=17).
7.4.3 Quality of life of child with SNI and impact on the family

Twenty of the parents who completed the PedsQL™ FIM, as outlined above, also completed the Caregiver Priorities & Child Health Index of Life with Disabilities (CPCHILD™) questionnaire on behalf of their child with SNI. CPCHILD™ is a validated measure of “comfort, health and wellbeing, ease of caregiving and quality of life of children with severe disabilities”.[613] It consists of 37 items distributed across 6 domains which are reported as standardised scores and a total score (each 0-100) with higher scores implying better QoL. CPCHILD™ scores for our cohort are shown in Table 7.3.

CPCHILD scores were correlated with the PedsQL™ FIM. Parental Physical Functioning correlated with the child’s Comfort and Emotions (p=0.0051), Overall QoL (p=0.0125) and Total score (p=0.0171)(Table 7.4). Social functioning correlated with Personal Care/Activities of Daily Living (ADLs) (p=0.0012), Overall QoL (p=0.0386) and Total score (p=0.033; Table 7.4). Emotional functioning correlated with Overall QoL (p=0.0059; Table 7.4). Cognitive functioning did not significantly correlate with any of the CPCHILD domains (Table 7.4).

Daily Activities and Family Relationships, both contributors to “family functioning” were found to correlate with the child’s Personal Care /ADLs (p=0.0038 and p=0.003 respectively) and total CPCHILD scores (p=0.0058 and 0.0155 respectively; Table 7.5). Neither Communication nor Worry were found to correlate with the CPCHILD scores.

The summary scores, i.e. PHRQoL, FFSS and Total score, were all significantly correlated with the child’s Personal Care/ADLs scoring on the CPCHILD™ (p=0.0149, p=0.0013, p=0.0127 respectively; Table 7.6). Parental HRQoL also correlated with their child’s Overall QoL (p=0.0084) and Total (p=0.0177) CPCHILD™ scores (Table 7.6). Total PedsQL™ FIM score was found to correlate with Overall QoL (p=0.0139) and Total (p=0.0142) CPCHILD scores (Table 7.6). Finally, FFSS correlated with their child’s Total score (p=0.0058; Table 7.6).
Table 7.3. Caregiver Priorities & Child Health Index of Life with Disabilities (CPCHILD™) scores as reported by parents of children with Severe Neurological Impairment (n=20). Items are reported as standardised scores (range 0-100) with lower scores representing poorer quality of life. Those domains which are non-normally distributed are shown as median (95%CI), while those which are normally distributed are shown as mean (SD).

<table>
<thead>
<tr>
<th>Domain – non-normally distributed</th>
<th>Median (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Positioning/Transferring/Mobility</td>
<td>29.17 (20.83-31.94)</td>
</tr>
<tr>
<td>- Communication and Social Interaction</td>
<td>26.67 (11.9-40.48)</td>
</tr>
<tr>
<td>- Overall Quality of Life</td>
<td>40 (40.00-60.00)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Domain – normally distributed</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Personal Care/Activities of Daily Living</td>
<td>31.99 (16.36)</td>
</tr>
<tr>
<td>- Comfort and Emotions</td>
<td>67.82 (23.82)</td>
</tr>
<tr>
<td>- Health</td>
<td>48 (16.31)</td>
</tr>
<tr>
<td>- Total Score</td>
<td>41.12 (12.17)</td>
</tr>
</tbody>
</table>
Table 7.4. Correlation of CPCHILD scores with parental health related quality of life scores (HRQoL) from the PedsQL FIM. ADLs, Activities of Daily Living; Significance was set at p 0.05 and significant values are highlighted in bold.

<table>
<thead>
<tr>
<th>Parental HRQoL (PedsQL FIM)</th>
<th>Physical</th>
<th>Social</th>
<th>Emotional</th>
<th>Cognitive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$ [95%CI]</td>
<td>$p$ value</td>
<td>$r$ [95%CI]</td>
<td>$p$ value</td>
</tr>
<tr>
<td>Child HRQoL (CPCHILD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal care/ADLs</td>
<td>0.3215 [-0.14 to 0.67]</td>
<td>0.1669</td>
<td>0.6724 [0.33 to 0.86]</td>
<td>0.0012</td>
</tr>
<tr>
<td>Positioning/Transferring/Mobility</td>
<td>0.146 [-0.34 to 0.57]</td>
<td>0.5509</td>
<td>0.3244 [-0.17 to 0.69]</td>
<td>0.1753</td>
</tr>
<tr>
<td>Comfort &amp; Emotions</td>
<td>0.63 [0.23 to 0.85]</td>
<td>0.0051</td>
<td>0.0183 [-0.45 to 0.48]</td>
<td>0.9426</td>
</tr>
<tr>
<td>Communication &amp; Social Interaction</td>
<td>0.2776 [-0.22 to 0.66]</td>
<td>0.2499</td>
<td>0.0594 [-0.42 to 0.51]</td>
<td>0.8091</td>
</tr>
<tr>
<td>Health</td>
<td>0.186 [-0.28 to 0.58]</td>
<td>0.4325</td>
<td>0.0024 [-0.44 to 0.44]</td>
<td>0.99</td>
</tr>
<tr>
<td>Overall QoL</td>
<td>0.5471 [0.12 to 0.8]</td>
<td>0.0125</td>
<td>0.4656 [0.015 to 0.76]</td>
<td>0.0386</td>
</tr>
<tr>
<td>Total score</td>
<td>0.5263 [0.11 to 0.79]</td>
<td>0.0171</td>
<td>0.4781 [0.05 to 0.76]</td>
<td>0.033</td>
</tr>
</tbody>
</table>
Table 7.5. Correlation of parental communication, worry and family functioning scores with CPCHILD scores. ADLs, Activities of Daily Living; Significance was set at \( p \leq 0.05 \) and significant values are highlighted in bold.

<table>
<thead>
<tr>
<th>Parental HRQoL (PedsQL FIM)</th>
<th>Communication</th>
<th>Worry</th>
<th>Daily Activities</th>
<th>Family Relationships</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Child HRQoL (CPCHILD)</strong></td>
<td><strong>r [95%CI]</strong></td>
<td><strong>p value</strong></td>
<td><strong>r [95%CI]</strong></td>
<td><strong>p value</strong></td>
</tr>
<tr>
<td>Personal care/ADLs</td>
<td>0.3386 [-0.12 to 0.68]</td>
<td>0.1442</td>
<td>0.0819 [-0.37 to 0.51]</td>
<td>0.7314</td>
</tr>
<tr>
<td>Positioning/Transferring/Mobility</td>
<td>0.3351 [-0.15 to 0.69]</td>
<td>0.1608</td>
<td>-0.2248 [-0.63 to 0.27]</td>
<td>0.3537</td>
</tr>
<tr>
<td>Comfort &amp; Emotions</td>
<td>-0.0236 [-0.49 to 0.45]</td>
<td>0.926</td>
<td>0.2324 [-0.26 to 0.63]</td>
<td>0.3534</td>
</tr>
<tr>
<td>Communication &amp; Social Interaction</td>
<td>0.2857 [-0.21 to 0.66]</td>
<td>0.2358</td>
<td>0.0439 [-0.43 to 0.5]</td>
<td>0.8582</td>
</tr>
<tr>
<td>Health</td>
<td>0.183 [-0.28 to 0.58]</td>
<td>0.4401</td>
<td>0.2681 [-0.2 to 0.64]</td>
<td>0.2531</td>
</tr>
<tr>
<td>Overall QoL</td>
<td>0.2248 [-0.26 to 0.62]</td>
<td>0.3407</td>
<td>0.1374 [-0.34 to 0.56]</td>
<td>0.5634</td>
</tr>
<tr>
<td>Total score</td>
<td>0.4183 [-0.03 to 0.73]</td>
<td>0.0664</td>
<td>0.1123 [-0.35 to 0.53]</td>
<td>0.6373</td>
</tr>
</tbody>
</table>
Table 7.6. Correlation of PedsQL FIM summary scores with CPCHILD scores. PHRQoL, Parental Health Related Quality of Life; FFSS, Family Functioning Summary Score; ADLs, Activities of Daily Living; Significance was set at p≤0.05 and significant values are highlighted in bold.

<table>
<thead>
<tr>
<th>CPCHILD</th>
<th>PedsQL FIM</th>
<th>PHRQoL</th>
<th>FFSS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r [95%CI]</td>
<td>p value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal care/ADLs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5358 [0.12 to 0.79]</td>
<td>0.0149</td>
<td>0.6665 [0.32 to 0.86]</td>
<td>0.0013</td>
</tr>
<tr>
<td>Positioning/Transferring/Mobility</td>
<td>0.2338 [-0.26 to 0.63]</td>
<td>0.3354</td>
<td>0.3419 [-0.15 to 0.7]</td>
<td>0.152</td>
</tr>
<tr>
<td>Comfort &amp; Emotions</td>
<td>0.3384 [-0.15 to 0.7]</td>
<td>0.1695</td>
<td>0.2356 [-0.26 to 0.63]</td>
<td>0.3467</td>
</tr>
<tr>
<td>Communication &amp; Social Interaction</td>
<td>0.2824 [-0.21 to 0.66]</td>
<td>0.2413</td>
<td>0.1952 [-0.3 to 0.61]</td>
<td>0.4233</td>
</tr>
<tr>
<td>Health</td>
<td>0.1051 [-0.35 to 0.52]</td>
<td>0.6593</td>
<td>0.0742 [-0.38 to 0.5]</td>
<td>0.756</td>
</tr>
<tr>
<td>Overall QoL</td>
<td>0.5722 [0.16 to 0.81]</td>
<td>0.0084</td>
<td>0.439 [-0.02 to 0.74]</td>
<td>0.0528</td>
</tr>
<tr>
<td>Total score</td>
<td>0.5241 [0.11 to 0.78]</td>
<td>0.0177</td>
<td>0.594 [0.21 to 0.82]</td>
<td>0.0058</td>
</tr>
</tbody>
</table>
7.4.4 Focus groups for teenage siblings of children with SNI

7.4.4.1 Demographics

Eleven families out of a total of 28 families were initially contacted and invited to participate. In the remaining families, the child with SNI did not have any siblings, or the siblings were outside the predetermined age-range of 12-18 years. Two of the families were known to have more than one sibling eligible to participate. Seven siblings were initially recruited for the focus group but only 4 presented for participation. The other three siblings declined, after initially agreeing to participate. The siblings were relatively homogenous in that they were all aged between 12 and 14, were of Irish nationality, had at least one sibling with SNI and were recruited from families attending a single paediatric complex needs clinic. There were no sibling pairs. The children with SNI ranged from ages 6 to 17. Diagnoses of the children with SNI included CP and rare genetic disorders. In order to maintain anonymity, further details of underlying diagnoses will not be elaborated upon here. Pseudonyms for the siblings are used throughout to protect the identity of the participants.

7.4.4.2 Thematic analysis

The analysis resulted in four interrelated themes: (1) Making sense of their situation today and tomorrow; (2) The sibling relationship within the family; (3) Personal impact for the siblings; (4) Encounters with healthcare professionals and support for the future. The themes are described below along with representative quotations presented with participants’ pseudonyms.

Theme 1: Making sense of their situation today and tomorrow

This theme explored how the participants attempted to integrate the idea of their sibling with SNI into their perception of a normal family life. The understanding of their sibling’s diagnosis and their personal re-interpretation of this was explored (Sub-theme 1.1. Diagnosis and condition related concerns). The need for a conventional family life through normalisation of their experience was uncovered (Sub-theme 1.2, Normalising) and the various narratives that siblings constructed for the outside world were examined (Sub-theme 1.3. Conflicts from the wider world).

Subtheme 1.1: Diagnosis and condition related concerns

This sub-theme represents the participants' understanding of their siblings' diagnosis. The siblings in this study were aged between 12 and 14 years, thus, their
understanding and comprehension were at a comparable level. Only one of the siblings were able to name their siblings diagnosis;

“I have a sister who has [XX] syndrome” Matthew

Two of the other participants showed a lesser degree of awareness and understanding of the name of their siblings’ condition. One of the siblings had an idea referring to ‘some kind’ of a disorder and another sibling was aware that his siblings’ did not have a name for their conditions;

“I’ve a brother with autism. Some kind of autism that I cannot name, I’m not sure…” Clare

“…we don’t really know what disability they have apparently so we can’t fully name it.” Sam

Interestingly, Emma never referred to her brother’s diagnosis throughout the focus group, but merely referred to his disability as being ‘in a wheelchair’;

“I’ve a brother … who is in a wheelchair.” Emma

The siblings all spoke openly about the differing needs of their brother or sister relating to their condition;

“he started like not being able to walk or anything when he was two. He was tube fed till he was four and then they put, then he got the PEG when he was six and he’s been with the PEG now for I think a few months.” Clare

“And basically she has [XX] syndrome which basically means she’s smaller and mentally younger than she actually is. So she’s six years old but she would act like she’s three. She’s in a wheelchair, she can’t walk, she can’t talk, she can’t do anything on her own, she’s deaf in one ear and she has epilepsy. And recently over the last week she actually came into the hospital because she just kept throwing up and we’ve been in the hospital, she’s still in hospital. And overall she’s not really in hospital that much but there’s nothing she can do on her own, either she’s constantly with my mum or my dad or one of her nurses.” Matthew

“My brother he’s like in a wheelchair he can’t exactly talk or walk or anything like that. But like it’s not that difficult for my parents like obviously at the start for them it was, but it’s gone easier and it’s not that hard like.” Emma
“Yeah, [siblings] they are both, well we presume they both have the same thing. They both are in wheelchairs they can’t speak or really do anything. And we don’t really know what they have.” Sam

“my nanny was giving him [brother] a treat and he can’t eat, he started choking and he was choking for about a minute and a half.” Clare

Three of the siblings spoke about “scary” experiences when their sibling had condition-related seizures. They provided detailed accounts and demonstrated a good level of understanding of what happened;

“Yeah, yeah I was there. It was like a good few years ago but like it was sort of, it was a very scary experience because we didn’t really know what was causing it to be so long, I remember it being like forty-five minutes so like way longer than it usually was. And like we called an ambulance and like they went into the hospital and they were fine afterwards, I think it was just sort of, well I don’t remember the cause actually but it wasn’t anything serious. But at the time it was quite scary.” Sam

“He was in hospital for a few months and we were told, we asked them to give him, I’m not sure what it’s called ECG or something to check if he had seizures. They did that but he was in an induced coma and so when they did that it came up clear but then I think it was a week after he got out of hospital he was in bed and we have a monitor that would say be like a security camera like, where you can see him. When he moves or makes a noise the screen goes on. So I don’t know but the screen turned on and I looked at it and he was like twitching and not normal so I went into him and he was having a seizure. The first time he had a seizure. My mum was in the kitchen and my dad was in the shower. I called and they came.” Clare

“Yeah, she was three years old so it was kind of scary but it wasn’t really surprising because throughout her entire life going from age one to age four she was constantly in and out of hospital for every, like she was in hospital at least once a month or so. So her having that one seizure it was scary and it was sudden but it wasn’t really shocking because we knew then that she did have epilepsy.” Matthew

To summarise, this sub-theme examined the siblings’ understanding of their brother or sisters’ condition. Only one participant had knowledge of the specific medical diagnosis relating to their siblings’ condition, with another participant clearly stating there
is no specific diagnosis for his sisters' condition, and the other two participants using
generic terminology to describe the nature of their difficulties.

**Subtheme 1.2: Normalising**

As part of the process of normalising, the siblings described their experiences in everyday
terms and the effect on their day-to-day life. Siblings often felt a disruption to routine due
to hospitalisations, which led to her becoming more independent from a young age;

“I feel like I'm getting used to it now, it's been like this since I was six, when I was six I got
to do stuff, he would come out of the hospital for say a month and then he went back in.
But now he's doing pretty good, and last year I think there was like, he was out of hospital
for like ten months and we were hoping he wouldn't go into hospital for Christmas
because he always went into hospital and yeah.” Clare

The siblings demonstrated an overall consensus of acceptance as a result of their brother
or sisters' condition;

“But if I want to do something with my friends and there's nothing on they like I'm normally
allowed to do it. So it's not really impacting on stuff I want to do.” Sam

However, some participants also focused on the activities they couldn't do as a result of
their siblings' condition;

“I don't really get to go out much, he needs loads of help but when I do I'm only out for like
half an hour max because I need to come back in to help my mum again.” Clare

“Sometimes when my mum is in hospital like I can't go swimming because there's no one
to drive me there.” Matthew

In summary, this sub-theme examined the way siblings normalised their experiences. This
was seen through a general acceptance of “getting used” to living with their sibling and
may have been due to their ages and understanding as adolescents, with feelings of the
disabilities “not impacting” on their lives.

**Subtheme 1.3 Conflicts from the wider world**

Here we explore how the siblings internalised their understanding of their brother
or sister’s condition, attached meaning to it, and subsequently managed their emotions
surrounding communication with friends and others outside the family. This sub-theme also details how much siblings are prepared to share with others. The analysis also illustrates the internal struggles that siblings have in managing how their brother or sister may be perceived by the outside world, with one sibling recalling a time when people 'joked' about those with disabilities.

Minimising attempts to disguise their sibling’s disability was a recurring observation, with all siblings reporting a reluctance to share their stories with others;

“I don't really explain it to people because its more I get to know them and then become friends with them and then if they happen to like see my sister then I'll explain it but I don't really need to do so I don't.” Matthew

“I would say I'd be the same, if they see my brother then I'd explain it but otherwise no, I wouldn't need to tell them.” Clare

“ Basically as everyone just said here if they have seen my brother then I'd obviously explain it to them. If they have any questions I would answer it for them.” Emma

“like you know when somebody comes up to you and asks oh you have a brother like this or a sister like this. So obviously it’s a bit scary telling them because you never know what they might say or feel about you or that.” Emma

“Yeah, like if I have a new friend normally, and they don't know beforehand, I would like sort of say… I would probably just tell them that like I have a sister with disabilities. And you know just tell them I guess. And like all the time like they would, they just say oh yeah that’s hard…” Sam

“And also I'd wait to be friends with someone to let them know that both my sisters have disabilities not like for any, not because I'm embarrassed of it or anything. Just because like if I met someone and one of the first things I said was oh my sisters are disabled, like it’s sort of a, like something, like most people wouldn't really know how to respond to it. Because it's not sort of a normal thing to meet someone who, well it's not exactly, well that was sort of worded wrongly but like most people I've met like I've never met someone with a sibling with a disability before. Most of them don't really know how to react appropriately. Which I mean if they reacted like wrongly I don't get mad obviously because if I was in the same situation I'd probably like react inappropriately as well. But like I'd normally just like ignore it.” Sam
One sibling spoke openly about how they feel when other people joke about people with disabilities, but although they were impacted by their comments, the sibling tried to justify why he thought they would make jokes;

“Like sometimes my friends or like some other people like they would make jokes about you know other like people, because of who they, in terms of disability and I know they don’t mean it, they are not bad people they are just making jokes. But I would not do that because obviously of my sisters and it sort of helped me be more empathetic towards people... Like I don’t mind people making jokes as long as I know like they are not actually you know labels or whatever you know which I mean they are not like.” Sam

However, despite feelings of discomfort when telling people about their siblings’ condition, all of the siblings appeared comfortable sharing information with their friends. All of the siblings discussed the importance of friends in their life, and telling them their story, with explanations given such as;

“They (friends) are very, very important to me but I actually really like hanging out with them.” Clare

With one sibling being able to relate to her friend due to a similar family situation:

“I actually have like a friend in school who has the same thing going with her family. So like we have more things in common and she can understand my situation and I can understand hers.” Emma

Clare and Emma enjoy “hanging out with friends” because it is “fun” and discuss finding it easier to talk to their friends rather than their parents;

“I…am not really able to talk to anyone but like my friends they understand it all, they are who I talk to.” Clare

“Yeah. It’s mainly my mam, my dad, me and two of my friends that know [brother] inside out. I’ve shared everything with my friends, just in case like I’m out or anything and my mam needs so much help.” Clare

“Yeah, just a little bit like my friends don’t live in my area so it’s a bit harder for me to hang out with them but when I get the chance to my mam and dad let me, so yeah.” Emma
The siblings were open with their friends about their brother or sister’s disability and comfortable with sharing in this arena;

“Like in primary school I was bringing like my friends' home for the first time and like they saw my brother and like they started asking questions obviously I didn’t mind because they were actually like one of my best friends so I explained it to them and they understood and like they didn’t mind or anything.” Emma

This sub-theme demonstrated that when siblings were not in a familiar environment, with family or friends, the norms were different, and siblings reported a reluctance to share information about their siblings’ disability with those outside of their inner circles. Siblings explored the importance of friendships and how they relied on their friends for a safe space to talk, rather than leaning on their parents.

**Theme 2: The sibling relationship within the family**

This theme captured the different ways participants’ experienced parental attention and how the sibling was situated within the family. This theme began with the analysis of how siblings built relationships with family members (Sub-theme 2.1. Family relationships) and then examined how their sibling relationships were shaped by the additional need to care for, and protect their brother or sister with SNI (Sub-theme 2.2. The nature of care, who does what; role, responsibility and function).

**Subtheme 2.1: Family relationships**

Sibling relationships were contextualised by wider family relationships and the relationship with their sibling with SNI were explained through relationships with other family members. One of the siblings reported having to constantly stay with other family members when their brother was in and out of hospital;

“But since I was three I was in and out of my uncle’s, my nanny and my granddad house and I was never home because [brother] was always sick, I had to rotate around the houses every week or two.” Clare

Siblings also reported the stress that is evident in their parents due to their brother or sister’s SNI;
“my parents were too scared. And he got it [PEG] a few months ago and he’s doing pretty good with it. Although for my mum it’s a lot of stress and like she doesn’t know when to feed him or when to put him on the feed and how long feeds last and everything.” Clare

“Just like pick it [stress] up like, I know my mam.” Clare

With one sibling highlighting their sisters’ SNI does not impact on him much, but has a great impact on their parents;

“It hasn’t impacted me a massive amount its mainly my parents that it impacts on the most. And I’ve been able to keep up my training...” Matthew

Siblings reported their parents being “constantly on the move” and busy with their sibling with SNI, affecting the time available for building their own relationship;

“My mum does everything...So she’s constantly on the move...... all the weekdays its early in the morning... And so usually my sister isn’t actually awake but whenever my mum is out it’s my dad that will take care of her. ” Matthew

“Yeah it did, like they already had a lot to do but they had to put them on a feed and stuff like that and make sure they were doing all right.” Sam

Following on from this reality of ‘busy lifestyles’, when asked if their parents had time to spend with the well sibling by themselves, all of the siblings, aside from one, reported relatively positive experiences with good support provided by their parents;

“Yeah, my parents tried their best for me to have a normal enough life. But like they, they sort of, they made sure that I was being looked after and like that I could talk to them and stuff. And like I had a lot of time to talk to them and stuff, and like my sisters would probably go to bed a bit early and then we’d be like sort of have time together and talk. So it was good.” Sam

“No, they have time, we do get to talk but not all the time. Sometimes we do.” Emma

“I mean I’ve never really needed a huge amount of support. Because my parents have been incredible throughout. Like answering questions and stuff like that.” Sam
Clare was the only sibling who spoke about never having one-on-one time with her mam or dad, but said;

“I don’t really mind it, I get that they have to do a lot. I don’t mind.”

When prompted and asked how her parents would manage if Clare wasn’t in the house to help and asked would they miss her, she simply said “I’m not sure.” Clare also reported there was “not really” anyone else who could help if she wanted to go out alone with her mam. Clare reported being able to talk to her grandmother, but did not expand further on that relationship.

In summary, this sub-theme explored the relationships and experiences between the well siblings, their parents and the overall impact their brother or sister’s condition had on the family dynamics. Within their responses, the siblings expressed a knowledge of their parents extra caring responsibilities.

**Subtheme 2.2: The nature of care, who does what: role, responsibility and function**

Siblings’ participation in caring for their brother or sister with SNI was a common theme throughout the focus group with varying levels of care expected of them. Siblings discussed helping out in the home with chores rather than actively participating in care for their sibling with SNI;

“Just do the dishwasher and clean the kitchen up and just make sure my siblings are okay and if they need anything I’ll just give it to them.” Emma

“Not necessarily, the only things that me and my brother get are just the very generic ones like empty the dishwasher, take the bins out, bring down laundry or dishes. We can just take care of ourselves. My younger brother is the same way but he has to have a bit more minding because he’s nine.” Matthew

Some siblings discussed participating heavily in care, alongside supporting their parents to care for their brother or sister and not being able to remember a time in their life when they were not helping out;

“If my mum isn’t able to if she misses a feed I know exactly what she would do so I’ll get that done. And if my mum needs, if my mum misses his medicine or doesn’t like, forgets to give him his medicine I’ll go and do that. I will give him his, I’ll put him in his wheelchair if he’s going out to the bus I’ll show them his medicine. And I’ll help him onto the bus. Now
I think it’s two years ago they were putting him on the bus and they left the suction machine down on the road. And they left off. And his, I’m not sure what they are called but some doctor he has they were coming to the school to visit him and he needed his suction machine and they were just left there on the road.” Clare

“There has to be someone in the room with [sibling] at all times and my dad is working so if my mum is not there my older brother or myself will be sitting with her the whole time.” Matthew

There were mixed experiences from the siblings, with some assisting in the day-to-day minding, and knowing their sibling “inside out”;

“Most of the time we leave her in her chair or if she wants to get out of her chair we put her on the couch and she’ll watch TV but if anything bad happens like she has a seizure or anything like that I’ll just ring my mam if she’s out and she’ll come home as soon as she can. And I’ll get my dad and brother.” Matthew

“He likes sitting in the garden in his wheelchair just like looking at the sky and everything and he also has a suction machine in case he coughs up and everything it’s not that hard.” Emma

“In my family not even my nanny or granddad knows him like I do, I know him inside out.” Clare

Others spoke about not having any caring responsibilities;

“No, not really. I asked my parents if they needed help regularly just in case but most of the time they said no. So I didn’t have any added chores or anything to do because like they could handle it.” Sam

Despite these caring responsibilities and increased chores, no siblings expressed difficulty in caring for their sibling nor discussed feeling resentment or anger towards their brother or sister. A immense feeling of a desire to help emerged and the siblings described positive changes including increased maturity and the development of empathy as a result;

“My brother he has a feeding tube so my mum has taught me it’s actually not that difficult as it looks. So yeah, it’s kind of it.” Emma
“For me, it’s not that; it hasn’t been that hard for me, like my parents encourage me to go outside and hang out with my friends. And like I can do a lot of things like they don’t make me do everything like help them with my brother, but it’s not that hard.” Emma

“Just like if my mam is going out somewhere with her friends and my dad is not at home all she tells me to do is feed my brother. It’s not that hard. And like it’s pretty normal for me it’s not that difficult. If we are getting ready for school will get ready for school on time, my brother and sister will get to school on time and everything so it’s not that hard.” Emma

“But like compared to a lot of other stories I’ve heard from siblings like I’ve been on the better side. Like I’ve been on the better side in terms of experience, like nothing terrible has ever happened apart from this one thing where they had a really long seizure but apart from that like, and they were fine after it, but apart from that like I’ve had a pretty good experience with them and it helped me mature a lot more than I would be if they weren’t both disabled. But obviously it’s a bad thing like.” Sam

“I’m so used to being around people with more needs than me. And I’d say that sort of helped me mature.” Sam

Some siblings reported assistance from other children within the family when caring for their brother or sister with SNI;

“my little sister she’s pretty nice she likes helps me sometimes, she’s six.” Emma

But others reported having other siblings who did nothing to help;

“She’s [older sister] really lazy, she won’t get a job… She just lies in bed all day on her phone.” Clare

Here we have explored the development of sibling relationships within the family unit. The sense that participants wanted to support their parents in caring for their sibling and happily took on extra chores to relieve pressure was evident. The notion of caring surfaced quite strongly in one participants’ account (Clare), whilst other siblings were less involved in the hands-on care provision. Participants spoke honestly about their experiences. All siblings showed a great awareness that their brother or sister required a higher level of care due to their condition, but no siblings discussed negative feelings towards their siblings or parents as a result of this. An overwhelming sense of acceptance
and cooperation was demonstrated. Despite this, living with a brother or sister with SNI did have an impact on certain areas of the siblings’ personal life.

**Theme 3: Personal impact for the siblings**

Despite a sense of acceptance of their siblings’ condition, and an adjustment to their new ‘normal’ life, there were elements of the siblings day-to-day lives that were affected. The two main sub-themes here, aside from care provision, as previously discussed, were school and holidays (Sub-theme 3.1. The effect on school and holidays), and the impact on daily routines including active participation in activities (Sub-theme 3.2. Changed daily routines). Due to the timing of the focus-groups, which were conducted during a time of pandemic-related restrictions, the siblings also spoke about the impact, or lack thereof, these restrictions had in relation to their daily lives living with a brother or sister with SNI (Sub-theme 3.3. Living in a world with COVID-19).

**Subtheme 3.1: The effect on school and holidays**

Initially, none of the siblings reported their schooling being affected due to having a brother or sister with SNI. However, after further exploration of this issue, some of the siblings began to explain how their sibling may affect their attendance at school;

“Kind of, because of where I live I’m about ten kilometres away from the town my school is in so I couldn’t walk there even if I wanted to. So if my sister was sick it’s kind of would have an effect because I take the bus but sometimes I might have to go to the hospital for like other reasons or might have to miss it entirely because my mum wouldn’t be able to be there on time.” Matthew

“No, I don’t think that they’ve ever impacted a lot…Impacted my school life a lot, I think the most would be missing out on a bit of homework but I never like, nothing major ever happened because of their disabilities.” Sam

“Not really, my education hasn’t been affected at all. Not for me or for my siblings, everything is okay.” Emma

Matthew also spoke about how understanding his school was;

“Yeah, they don’t mind if you are late, as long as you are there and they don’t mind if you leave early either as long as you have like a proper reason to be late or leave early.”
Conversely, siblings felt a huge impact on holidays as a result of their situation. All siblings felt that holidays were an important issue to discuss, with an obvious impact caused by their sibling with SNI;

“I don’t go on holidays because of everything” Matthew

“Yeah and also I’ve never been out of Ireland with my sister because I’ve been out of Ireland once to go to the UK…. And that was [before his sister was born]” Matthew

“When he was four we went to Disneyland, or when he was two but he was really bad there, so we didn’t go, we went to England a few years later. So after that we gave up on going outside, we… we hmm… I forget now… we went to Trabolgan just like to see; it was quiet there but other than that there’s nowhere we can bring him.” Clare

“My family they live in the UK and my uncle’s family so my brother can go like on the ferry. On planes it’s a bit more difficult, but like if we are going to the park we can easily bring him or shopping sometimes. Like going to the airport or going to another country it’s a bit more difficult. So like we basically would be to houses or something like that, so yeah like we’d leave him with a nurse. Only for a small amount of time though like a week.” Emma

“In my case we used to get respite for them but that did like limit us to at most a week or two weeks away.” Sam

Despite these restrictions associated with life with a sibling with SNI, the siblings spoke with a resigned ‘acceptance’ about things they could or could not do.

**Subtheme 3.2 Changed daily routines**

There were mixed feelings about whether the siblings with SNI affected the well siblings in daily life. One participant did not feel their siblings hindered their activities at all;

“Normally we would bring them with us, I mean like then we would only go to places that they could go with us. there’s this place, I can’t remember where it is … it’s like a huge garden because they love sort of plants and stuff. So we used to go there and they loved that.” Sam

In contrast, another was very restricted by their siblings condition;
“He has very, very bad social anxiety so if we bring him outside and there’s any sort of noise it’s hands up to his head and he would be shouting. And so we are not really able to go outside, but he loves just sitting in the back garden, looking at the birds.” Clare

When talking about their daily routines, the accounts given by the participants very much revolved around their siblings’ care and needs, rather than placing the well sibling themselves at the centre of their day, which is reflective of an understanding that life revolved around the child with SNI;

“We would, as soon as we get up we would put his feed on and when he starts feeding we give him 16mls of water and then his three antibiotics. And then he gets three feeds a day, his last one starts at 6 and ends at 8, and then he has six different medicines. None of them work. But the doctors are still telling us to give them. Nothing is different with him; nothing works for any of his medicines or anything. I’m pretty sure before he went into hospital to get the PEG, the reason he got the PEG was because he had an aspiration and he went to get sick but the mucus went back down into his lungs and burnt his lungs. So he was in hospital for a while then and I was rotating between two houses again. I didn’t really have anything to do, I couldn’t go out with friends or anything because I was still rotating. And I think he was two months in hospital just from his aspiration. We didn’t know what was going on for him, it was like half an hour and he was slowly but surely like losing his breath. So we called an ambulance.” Clare

“My personal routine, it’s not much different, it’s getting up and getting dressed and getting food, brush my teeth and go to school. But my parents, my mum or dad has to get up at seven to make sure everything is done and everyone is dressed. And my sister usually gets up around eight and at that point they go downstairs and I think she gets her medicine at nine o’clock. She’s on a lot of different medicines I’m not sure exactly but they are for different things. I remember about three years ago she; I don’t know exactly what happened but I think she wasn’t on a certain medicine just yet but she had a seizure that lasted about two hours… And eventually we got medicine for that, and proper like knowing what to do now. But she’s constantly on different medicines they are constantly cycling. There’s not a direct one that she’s on all the time because she can be on one for about six months and then get off that and go to another one. So she would get her medicine around nine which would be the same time she gets her breakfast. And she goes to school I think half nine or ten and get home from school I think at about one. And then she just sits in her wheelchair and watches TV and eat food and make noise. And she gets more medicine at around ten
O’clock at night and there’s not an exact time when she has her medicine I think it just has to be spaced out at certain times. So if she has her medicine at ten in the morning she would have to have her one at night at eleven. There’s not much to say, just pretty generic day.” Matthew

siblings spoke throughout the focus group about enjoying spending time on their own and when asked if they had someone to talk to the response was;

“There probably is but I’m one of those people that likes to be left alone most of the time, all the time I just spend in my room and just alone with my cats playing video games and don’t really talk to anybody even playing on my games I don’t really talk to anybody.” Matthew

The male siblings in the focus group spoke about their hobbies as mainly participating in solo activities with Matthew being a;

“swimmer… I train six hours a week” with a “full home gym”

Sam also reported liking “swimming” and going for;

“walks some nights with my dogs, I play like online with my friends. And I like to read, actually I’ve been reading since I was about two”

Matthew also reported that he didn’t “really do much just sit in my rooms and play games” and “I have two cats as well”.

The female siblings in the group did not talk about additional hobbies outside of spending time with friends. Within this discussion around hobbies, siblings discussed the constraints resulting from COVID-19 restrictions, which will be explored in the next sub-theme.

Subtheme 3.3: Living in a world with COVID-19

Due to the timing of the focus groups, with COVID-19 restrictions and reoccurring lockdowns in place, this concept of ‘living with COVID-19’ emerged within some aspects of daily life, including hobbies, schooling and family life. Matthew discussed missing out on “important [sporting] competitions” as a result of COVID-19, but nothing related to his sibling with SNI;
“Yeah kind of, usually once a year with my… team we would go to somewhere in Ireland just as a relaxing outing, just out and about. And this year we couldn't do that because of COVID so last year we went to [an adventure camp] and my older brother he is also… on multiple teams… and usually he would go to Spain once a year but he couldn't because of COVID.” Matthew

The siblings all discussed their experiences of online learning due to school closures as a result of COVID-19;

“it wasn't really that challenging, I'd do it whenever I had any spare time.” Clare

“Yeah most of our classes were live classes as well, and it was hard for me not necessarily because of my sister but just because I get distracted by everything. And I have a relatively big house, so finding room is never an issue. Because my sister would be downstairs and I'd be upstairs in my room.” Matthew

“Not really, and even like if one of them was in the house I'm in it’s a relatively big house because we moved there because we needed more rooms for them. So it was literally especially designed for them. So like we’ve a good amount of room and it wouldn't really affect me. But things in the house it didn't affect me at all.” Sam

“It's been okay, like I get to school on time and online school has been pretty easy. Just getting really distracted because I'm at home and like you are muted and your camera is off you could basically do anything. The teacher wouldn't even know.” Emma

Some siblings reported difficulties with online learning, but due to younger siblings, not related to the sibling with SNI;

“If my other brother wouldn't stop running around screaming yeah,” Clare

When the siblings were asked about any additional experiences and challenges of living with COVID-19 the results were mixed;

“Yeah like in lockdown last year me and my family used to go on a lot of walks in the park and everything, so I still do that today and everything. Going on walks, clearing my mind.” Emma
Interestingly none of the siblings found it more difficult having a sibling with SNI during lockdown as a result of COVID-19;

“No, not really.” Sam

“No really.” Clare

This theme has delved into the personal impact on the siblings, exploring important concepts in the lives of adolescents including schooling, holidays, day-to-day life and hobbies. The experiences shared by these siblings about their impact on daily routines have subtle similarities alongside stark differences, highlighting the idiographic nature of the analysis.

**Theme 4: Encounters with healthcare professionals and support for the future**

Siblings are an integral part of the family unit and their encounters with their brother or sister with SNI have been illustrated in the previous themes. This theme reflects on encounters with healthcare professionals and care provision for their sibling to date (Sub-theme 4.1. Encounters with healthcare professionals) and searches for answers to provide ways that things could be improved for other siblings in similar situations (Sub-theme 4.2. Looking to the future).

**Subtheme 4.1: Encounters with healthcare professionals**

There were very mixed experiences with healthcare professional encounters. There were some negative experiences, frustrations and feelings of being talked down to. These experiences concerned interactions with and understandings of medical professionals with regards to medication regimes. Siblings often felt like their brother or sister were on multiple medications which did not appear to work;

“I'm not really sure I feel like why would you put him on, leave him on it medicines as a test subject… only one of them works which is his seizure one” Clare

Within this context, Clare also reported that it seems;

“like they [medics] are trying but they just don’t seem to know”

Matthew also reported witnessing challenges with his sibling's medication regimen, issues within the care provided in hospital and his family not being listened to;
“Yeah but it’s not even that there’s not a good one, it’s that that good one only works for a certain amount of time before she eventually gets used to it and then it stops… It stops working and then you have to start again by going to another one and another one and another one.”

“Because growing up my mum she wanted to be a veterinarian and so she knows a lot about biology and a lot about what’s meant to be done so she knew exactly what my sister was going in for. And basically when she had that seizure we went to hospital and they didn’t give the right support and yeah just ended up in a mess. So we ended up getting transferred to another hospital.”

“It was frustrating because we went in knowing what we were doing and what we were going in for. And they just wouldn’t listen because my mum is not a doctor so apparently she doesn’t know what she is talking about.”

Clare also reported feeling angry “sometimes” with healthcare professionals and scared when she was unaware of what was going on;

“We were on to the hospital that did the ECG I’m not sure what it’s called… And then… they said they couldn’t do anything about it and that they were harmless and hung up the phone immediately. I’m not sure what hospital it was, but they were actually being very, very uncaring.”

“I wasn’t really sure what was going on although I did know he got sick, and it went back down into his lungs. They couldn’t figure out what was going on with him for about a month when he was in hospital so he had to stay there for another month or two. I was actually pretty scared, you know, when I heard.”

When probed to ask if Matthew can remember any good experiences in hospital or a time when he felt supported, his response was;

“Probably is but I can’t think of any off the top of my head.”

Conversely, Sam reports a more positive experience, despite some “small mistakes” in care delivery;
“Apart from the long seizure I haven’t really had any like scary moments. Hmm…I’d say the support the girls get in hospital and my parents get is pretty good. At some points there has been some small mistakes which I had forgotten because they haven’t been too bad but overall it’s been like good.”

“But apart from that, in terms of hospitals I think I get all the support over the years I’ve got the support I need.”

Similarly Emma, reports a positive hospital experience for her brother;

“Not too long ago like my brother came into hospital because he needed oxygen, so like the doctors wouldn’t let him go until the oxygen machine would come home. so that was a bit scary because he stayed in hospital for I think almost two weeks and my mum was in and out of the house and it was at the time where schools were closed. I think during March and April that time so that was the time he was in hospital, but thankfully they brought the oxygen machine home and now he’s okay. But it was a bit scary because he needed oxygen more than ever at that time.”

Emma also reports feeling supported during this time by her “mum and dad and the doctors and nurses” and said that she could gain information from “Probably like from a doctor like sometimes a nurse would come around and just cheer up people.”

Experiences of hospitalisations varied greatly among siblings, but this was one area that the siblings felt passionate about sharing, alongside their suggestions for what could be done better.

**Subtheme 4.2: Looking to the future**

Three of the siblings felt strongly about providing recommendations for other siblings in their situation. When asked about how things could be improved for siblings of children with SNI, the siblings had some suggestions they wished to share including support groups, siblings and family camps and online groups;

“I can’t exactly remember the name but it’s this camp that like only takes siblings of disabled children, I think, I can’t remember the name at all but I went there once and like I didn’t really want to go. But it was actually quite fun, because I mean they had like really nice activities and stuff like that. And obviously all the kids had siblings who had disabilities so like I made some friends there because we had stuff in common and stuff like that. It was quite good.” Sam
“I mean maybe an online group… But I would say for some people that maybe a group to talk to and ask questions and stuff would be good because not everyone has the amount of support I do. So maybe an online group would be good.” Sam

“I’d say an online group because it’s one of those things that you can attend whenever you really want to. But if it was in person you’d have to go and travel there and you might not be really comfortable because you are meeting new people whereas online you would see anyone or you wouldn’t hear anyone you’d just be there, and you know that everyone there would have something in common but you would have to listen to them. And you could just attend it whenever you really wanted to or if you don’t want to you don’t have to.” Matthew

In terms of support in the home, only one sibling, discussed home support for their sibling;

“And recently, I mean like yesterday, what you call it, this, this girl named [XX] came into our house she’s I think twenty-eight and she specialises in autism and people with disabilities, babysitting them and everything. So she’s going to be helping us.” Clare

One other sibling, Sam, talked about respite, which allowed them to go on holidays.

Interestingly, one sibling, had no suggestions about things that could be doing differently or better stating;

“I don’t know” Clare

All siblings were in agreement about the advice they would give to other siblings starting out on their journey;

“Just know that everybody understands your situation …..Just know that nobody is going to judge you and feel comfortable.” Emma

“I was going to say the same thing but I don’t, there’s nothing else I can think of.” Sam

With Matthew and Clare agreeing that there was nothing else they would say.

To summarise, here we have provided an analysis of the experiences of four young people who had a brother or sister with SNI. The experiences were clustered into four master themes, and within each master theme, different sub-themes were identified. The
analysis has captured something of the lived experience of these siblings. Within the analysis, commonalities between the siblings' experiences were combined and differences were highlighted.

7.5 Discussion

In this study we have demonstrated the significant impact that caring for a child with SNI can have on the family. We used the PedsQL™ FIM to assess parental wellbeing and to quantify family impact as compared to controls. We found that caring for a child with SNI has a significant impact on overall PHRQoL. This is in keeping with similar research in families of children with metachromatic leukodystrophy and pontocerebellar hypoplasia, conditions which lead to severe, chronic neurological disease (596). Physical and social functioning were significantly impaired in parents of children with SNI compared to controls. Emotional and cognitive functioning were not significantly different between the groups. Yao et al reported similar effects on physical and social functioning in parents of children with spinal muscular atrophy (SMA; n=101), which became more pronounced with greater levels of motor impairment (614). In contrast to our study, they also demonstrated poorer emotional and cognitive functioning in parents of children with SMA.

We demonstrated that communication is a particular problem for the parents of children with SNI. This relates to communication with healthcare professionals but also with wider society, with a major component of this domain asking whether the parent feels that others "understand [their] family's situation". In the Chinese context, parents of children with SMA did not experience the same communication difficulties as the parents in our study (614). However, a study by Rodocanachi Roidi et al described parental dissatisfaction with the provision of information by healthcare providers regarding their children with Rett syndrome (615). It is vital that we strive to improve communication with parents of children with SNI, not just for humanitarian reasons, but because better doctor-patient communication is associated with better objective health parameters and reduced healthcare costs (616).

Worry appeared to have a negative impact on the parents of children with SNI in our study. This worry not only relates to their child’s condition, its treatment and their future, but also to the impact that their child’s disability is having on other members of the family and how others perceive their child. Similar findings are described in families of children with special needs (n=84) in South Florida (617) with parents worrying much of the time. The most significant worry, reported by 86% of parents in the latter study, is regarding their child’s future (617). A similar study in Poland in children with CP demonstrated that worry was the most severely impacted domain in the PedsQL™ FIM (618), in keeping with our findings that worry has a significant impact on parents of children with neurodisability.
Overall, family functioning in the SNI group was significantly impaired compared to the control group. Both daily activities and family relationships were impacted but the impact on daily activities appeared to be especially severe. Our results are consistent with the wider literature which shows that children with chronic health conditions, including CP, have a significant impact on the daily family routines (617-619). Given the previously discussed results, it is not unexpected that the total family impact score was significantly lower in the families of those with SNI than in the control families. It is clear that almost every facet of the parents’ and family’s life is impacted. Our findings suggest that parents have many unmet physical and emotional needs which are creating a significant impact on the family.

In view of the considerable family impact reported by the parents of children with SNI, it is noteworthy that there were no significant differences between groups in almost any of the PedsQL™ scores. Physical, emotional, social, and total scores were similar between siblings in the SNI and control groups. This contrasts with findings by Kelada et al that siblings of children with chronic conditions had poorer psychosocial functioning on the PedsQL™ (620). However, that study compared the scores gathered from their study population with “published norms”. This may affect the reliability of the results as the “chronic illness” group were compared with controls which were separated from them in time and place. Another study reported no significant reduction in PedsQL™ scores amongst 37 siblings of children with intractable epilepsy (621) which is more in keeping with the results we have presented here. However, although the families recruited in the latter study had a significant chronic neurological condition, the majority were ambulant and did not have any co-existing medical conditions, implying that they were less medically complex with less functional motor impairment than the children with SNI in our study. Published norms were also used for comparison purposes in this study, so direct comparison with our results should be viewed with caution. We are also mindful that questionnaires may be too crude a measure to pick up subtle differences in wellbeing between siblings of children with SNI and controls. Considering that previous literature has shown that effect sizes in QoL measures for well siblings are small (344), this observation may have a significant bearing on our findings. Also, as was so keenly perceived by some of our teenage participants, the PedsQL™ questionnaire, in isolation, cannot fully capture the complexity of their experience.

Perhaps surprisingly, siblings of children with SNI reported better school functioning than controls. These findings contrast with a study by Mazaheri et al who showed lower school functioning amongst teenage siblings of children with Prader Willi syndrome (622). However, their study did not find statistically significant differences in psychosocial functioning which is comparable to our results. The better school functioning scores in siblings of children with SNI may be because of the timing of data collection in
relation to the COVID-19 pandemic. Although all of the data described in this study was gathered after the onset of the pandemic, control data was mainly gathered towards the end of the study period. In contrast, in the SNI group, questionnaires were administered over a longer, more diffuse, period of time. It is therefore possible that controls had poorer school functioning as many of them had been exposed to a more prolonged period of home-schooling than their peers in the SNI cohort.

We did not collect a control sample to compare with the CPCHILD™ scores of children with SNI. It is, therefore, not possible to comment on their wellbeing based solely on these scores. QoL is a highly subjective experience and values that one person/family may consider unacceptably low may be considered acceptable by another. That said, almost all domains in the questionnaire had a mean/median score less than 50 for the children with SNI, out of a possible maximum score of 100. We correlated the CPCHILD™ scores with the PedsQL™ FIM scores in an attempt to understand which aspects of the life of the child with disabilities had the most significant impact on their carers and immediate family members. Unsurprisingly, parental social functioning correlated with the child’s personal care and ADLs and the parent’s emotional functioning, daily activities and family relationships were linked with their child’s overall QoL. Less expected was the association between a child’s comfort and emotions and their parent’s physical wellbeing. Also, one may have predicted that CPCHILD™ scores would correlate with parental worry, but this was not the case. Overall summary scores in PedsQL™ FIM correlated with the overall scores in CPCHILD™ implying a substantial link between the QoL of the child with SNI and the wellbeing of their family. Our findings are similar to a study by Koltuniuk et al which showed a significant association between their child’s quality of life and the level of life satisfaction of the parent(618). In particular, they described how a greater physical and school functioning in the child was positively associated with parental life satisfaction. This may draw parallels with our findings that better scores in a child’s ADLs were associated with higher emotional scores in parents.

In gathering data from parents and young people, we engaged in several discussions with families who care for children with SNI. It became clear that teenage siblings, in particular, did not feel that questionnaires could adequately capture the complexity of their situations. It was as a result of these conversations that we created a focus group to provide teenage siblings with a forum to discuss their unique experiences of having a brother or sister with significant neurodisability. In the latter part of this chapter, we have used 4 main themes to interpret these lived experiences. Siblings experienced having to adjust to their family’s situation, including differences in the sibling role and position within the family and within society.

In this study, we have described how siblings attempt to make sense of their situation. A number of parallels exist in the wider literature about the effects of childhood
chronic illness. Positive and negative experiences are reported including adopting a “caring role”; increased autonomy and independence; greater compassion, sensitivity, and empathy; demonstrating maturity and responsibility; and reduced parental attention as a result of their sibling’s condition (620, 623-626). Similarly, all these elements have been reported by the siblings in this study.

Siblings in this study reported an overwhelming sense of acceptance and willingness to participate in care, which is consistent with a meta-synthesis conducted by Deavin et al exploring the experiences of siblings living with a child with a chronic illness (627). Deavin et al described that siblings altered their behaviours to meet their own needs and to take on additional roles with the family, taking on new tasks and skills (627). The adoption of new roles within the family was demonstrated by some siblings in this study e.g. learning new tasks such as suctioning or enteral feeding. The insertion of a feeding tube is largely viewed in the literature as having a positive on the child’s quality of life due to improvements in ease of feeding and medication administration for children with neurological impairment (392, 628). However, one sibling in this study reported it as a scary time for her parents. None of the siblings spoke about their own experiences or feelings around the time of the placement of an enteral feeding tube.

The siblings in this study all reported on daily treatment regimens, including medication management, seizure management and respiratory care, but did not associate any negative impact with these regular, on-going interventions, instead accepting them as part of day-to-day life. Conversely, previous literature has described the negative psychological effects of having a sibling with a chronic disorder. These negative effects were particularly marked where the condition had an impact on daily routines (629). Similarly, a negative psychological impact has been described in siblings of children with epilepsy who have regular seizures (630, 631). Interestingly, although the siblings did not report their sibling’s treatment regimes in a negative light, they did comment on some frustrations with the effectiveness of many of their medications. The siblings perceived uncertainty surrounding medication choices (“don’t seem to know”, “test subject”), delays in finding appropriate medications (“eventually we got medicine for that”), poor medication efficacy (“only one of them works”, “only works for a certain amount of time”), and continuous changes to medication regimes (“constantly on different medicines”, “going to another one and another one”). It would appear that these young people are acutely aware of the difficulties in effectively managing their siblings’ symptoms. Indeed, it is true that therapeutic trials are often utilised in clinical practice, and that our understanding of symptom management is rapidly evolving but there is still much uncertainty.

Communication is central to addressing these issues, and it may be useful to engage siblings in discussions around the complexity of treatment choices and goals of care.
None of the siblings in our study have described any negative feelings towards their brother or sister with SNI, nor did they share any attention seeking behaviour. Rather, they demonstrated a maturity and a sense of being responsible within their position in the family. Siblings discussed being more resilient than their peers with a greater ability to cope. In contrast, the concept of reduced parental attention in the context of the increased demands of caring for a child with a chronic condition are described in the literature, with a subsequent feeling of being left out by the well children(626, 632). The study by Tregidgo et al, as well as others describe feelings of resentment, anger, frustration, loneliness and resultant “acting out” behaviour(598, 626, 633). The siblings in this study did not report any of these emotions or feelings, nor did they share any episodes of acting out. This may be due to the age of the siblings, and a better understanding of their family situation. Alternatively, it could be because the siblings did not want to reveal any negative behaviours in a group setting.

The siblings in this study appeared at ease in their own company, enjoying spending time on their own, with many hobbies reported as ‘solo activities’ and not requiring input or participation from others. Despite this concept of enjoying spending time on their own, the importance of friendships was an emerging theme in this study. Overall in this study, siblings felt comfortable talking to their friends. Some siblings were more comfortable sharing their situation with their peers than others, which was reflected by their unique contributions. The importance of friendships in providing emotional and practical support is well described in the literature(623, 634-636). So too is the internal conflict arising from telling other people, aside from friends, about their sibling’s illness due to potential stigma(634).

The focus groups took place at a time when the COVID-19 pandemic was causing major disruption to daily life. The siblings in this study reported that lockdowns had “not really” affected them. Other studies reporting parents experiences of lockdown with children with intellectual disabilities and autism described worsening of behaviour and mental health(637, 638) which would, undoubtably, impact on family functioning. Indeed, Stoeklin et al described the impact of lockdown on physical, social and emotional health of children(639). No studies explicitly focussed on the wellbeing of siblings of children with neurodevelopmental disorders so it is difficult to directly compare our findings with the broader literature. It may be that the siblings in our focus groups did not see an impact because the social distancing led to a reduction in other circulating respiratory viruses(640), with the result that their sibling with SNI was less likely to require admission to hospital. Perhaps, the reduction in social and sporting activities, as well as the requirement for many parents to work from home, allowed the parents more time to spend with their children, thus buffering against some of the associated negative impacts of the pandemic. Without further research it is impossible to say why the siblings who
Some siblings spoke about times of “being scared”. These related to times where their brother or sister with SNI experienced a change in their health condition, e.g. a prolonged seizure or admission to hospital. Siblings recounted difficulties present within the hospital setting including times where they felt excluded by healthcare professionals. They felt that they had not received appropriate information, that their parents’ concerns were not heard or that their parents were not recognised as the rightful experts in their siblings’ care. Similar findings emerge in the realm of complex care where parents are often not given acknowledgement by healthcare professionals for their wealth of information and unique experience with their child’s condition (641). An integrative review of healthy siblings of children with cancer found that healthcare professionals should include siblings in the assessment of the family unit’s adaptation to the diagnosis and provide interventions to promote their psychosocial wellbeing (642). This recommendation is consistent with the recommendations of the young people involved in our focus group.

Siblings in this study suggested ways in which others in their situation could be better supported in the future, through therapeutic camps and support groups. These recommendations are consistent with wider literature in this area (627, 643, 644). Therapeutic camps have been reported by siblings of children with special health care needs as beneficial (644). A sense of family togetherness, improved peer relations, and enhancement of relationships between family and professionals were reported through the use of family camps (643). They have been described as enjoyable and somewhere where the young person can experience a place of belonging (645). Only one of the siblings in our study had experience of participating in a family camp, but similar results were reported, of ‘having fun’ and making new friends.

The use of support groups was suggested as another way to better support siblings of children with SNI. This is consistent with other studies which report support groups as helpful for siblings (365, 627), by allowing them to express their feelings in a safe space, positively affecting well-being and self-esteem (365). In keeping with the inclinations of the young people in our study, a preference towards online support groups was reported in another study of siblings (n=91) of children with chronic conditions (646). These recommendations are crucial to the development of resources to improve the overall experience of having a sibling with SNI.

There are several limitations in the current study. Our sample size, for the questionnaires, in particular, was relatively small. However, our sample represents a select population of families with exceptionally high healthcare needs and our findings are largely in keeping with the previously published literature. The participants were all recruited from the same hospital clinic which may impact on the experiences that the
families have had, especially their encounters with the health service. For the sibling PedsQL™ questionnaires, we relied on the children and young people completing them at home and returning them by post. One may question whether we can be certain that they were completed without undue influence from their parents, whether intentional or unintentional. There may also be an element of response bias in that those who responded may have particularly positive or negative views which they feel compelled to express. Fathers were significantly under-represented in the responses that were received, a trend that is, sadly, common in similar research (647). Future research in the area may benefit from explicit recruitment of fathers as their experiences are likely to differ from those of mothers (341).

Survey tools provide a relatively limited view of wellbeing in what is, in reality, a complex and multi-faceted concept. They also only offer a “snapshot” in time. On the other hand, the tools used here are well validated and are used extensively in similar literature. In addition, to add richness to the exploration of wellbeing in these families, we conducted a focus group with teenage siblings. The focus group captured the experiences of four children within a narrow age-range, whose voices are not often heard in research. Given the small sample size, the findings cannot be generalised to all siblings who have a brother or sister with SNI. However, the aim of IPA research studies is not to provide generalised findings, but to provide an in-depth exploration of each participant’s experience. This small sample size allowed these rich experiences to be reported. However, these experiences are unique to these four siblings and their individual family situations and others may describe different viewpoints. It is possible that those who declined to participate were those who had predominantly negative thoughts or were especially badly affected by their sibling’s SNI. Therefore, they may have felt uncomfortable, embarrassed, or guilty about sharing their experiences. In some cases, parents declined to consent to their child’s participation, due to fear of provoking anxiety or discomfort or because they “knew” that they would not be interested. This may have created a selection bias, in that those who participated may have been more likely to have positive experiences, or, to be from families where disability and wellbeing were openly discussed. It is difficult to avoid this potential bias in research of this type. Three siblings had agreed to participate but did not present themselves on the day the focus group was to occur. No alternative data collection method was offered to these siblings to ascertain their experiences, which is something to consider for future research.

Despite its limitations, the findings of this study have expanded the current knowledge and understanding of the wellbeing of families caring for children with SNI. It is essential that we understand the implications of caring for a child with SNI on the whole family. Current services are woefully inadequate in supporting parents as they provide support to their children, both those with and without a disability. There is an urgent need
to recognise siblings as an important part of the family unit and involve them in care delivery, in an age and developmentally appropriate manner. In the hospital setting, implications include the provision of information and support for these siblings. The siblings in this study recommended the use of online support groups and family therapeutic camps as ways to improve the experiences for siblings living with a brother or sister with SNI. Further research into siblings of SNI of different age groups will be beneficial to continuously improve care for this unique population.
Chapter 8 - Discussion
8.1 Introduction

Children with Severe Neurological Impairment (SNI) represent an important group of children who have complex medical needs. Children with neurological special healthcare needs have more unmet needs than children with non-neurological conditions, and this is especially true for children with multiple neurological conditions (2). Children with medical complexity (CMC) make up a small proportion of the population but account for a substantial proportion of healthcare costs (539). The value of grouping children with SNI together, as distinct from those with neuromuscular disorders or neurodisability, lies in the common and unique issues that children with disorders of the central nervous system face (3), as well as the fact that children with greater neurological impairment are more likely to experience other health-related issues (491). The lack of a universal definition of SNI may hamper communication and the value of research in the area.

Children with SNI may experience dysfunction in almost every organ system. To date the extent of this multi-organ dysfunction has not been quantified. Children with cerebral palsy (CP), who make up a significant proportion of those with SNI, have reduced life expectancy, with respiratory causes noted as one of the main sources of morbidity and mortality in this population (24, 25). Other systems, such as the gastrointestinal, cardiac, renal, immune, and musculoskeletal systems are also known to lead to significant morbidity in this cohort (491), and there may be value in considering CP as a multi-organ disorder.

The immune system and inflammation are thought to be involved in the pathogenesis of several neurodevelopmental disorders including CP (326) and autism spectrum disorder (498). It is also hypothesised that inflammation may sensitize the brain to further future insults and cause tertiary neurological injury (257, 327, 498, 538). Altered inflammatory responses are known to persist in children post-neonatal encephalopathy (NE) (127) and cytokine dysregulation has been demonstrated in children with CP (429). In addition, children with neurological impairment are at increased risk of infection related morbidity and mortality.

Given the increased care needs of children with SNI, there may be a substantial effect on the wellbeing of the families that care for them. There may be additional social, emotional, and financial pressures which may have an impact on family relationships and everyone within the family. Relatively little research exists in the area. In order to be able to provide holistic care to these families, it is essential that we understand their needs as much as possible.

In this study, we have chosen to focus on several aspects of the care of children with SNI. In chapter 3 we described issues around the definition of SNI, including the creation of a consensus-based definition. In chapters 4 and 5, we examined dysfunction in cytokine, inflammasome, and leukocyte responses following exposure to
lipopolysaccharide (LPS). In chapter 6 we have commented on renal and cardiac function in children with SNI as well as the extent of multi-organ dysfunction (MOD), proposing a preliminary MOD scoring system for children with SNI. Finally, in chapter 7, we described the family impact of caring for a child with SNI, focusing on their siblings.

8.2 The definition of SNI

In chapter 3, we aimed to describe the variability in the use of the term SNI and to place it within the context of the many other terms used to describe children with complex chronic health conditions in the literature. We then used the systematic, consensus-building Delphi process to create a multi-disciplinary, multi-national definition of SNI.

In the preparatory phase, we performed a comprehensive literature review, and demonstrated the wide variability in definitions of SNI, both those that were implicitly and explicitly defined(353). Several fundamental differences in definitions were evident, including with regards to motor, cognitive, communication and care considerations. Variability in definitions can be detrimental to communication in the research and clinical setting(421). We recruited a multi-disciplinary, multi-national panel of experts to participate in a Delphi process to further develop this concept.

The Delphi process is a well-recognised tool for reaching consensus amongst a group of experts. It is highly adaptable, anonymous, cost-effective, does not require special expertise to administer, and has been used numerous times for the creation of medical definitions(5, 14, 350). After 3 rounds, consultation with parents, and presentation at an international conference, we proposed the following definition for SNI which has been published in a peer-reviewed journal:

“Severe Neurological Impairment describes a group of disorders of the central nervous system which arise in childhood, resulting in motor impairment, cognitive impairment and medical complexity, where much assistance is required with activities of daily living. The impairment is permanent but can be progressive or static.”(428)

One of strengths of the definition lies in the structured method of consensus building. We chose the Delphi method for several reasons as outlined above and, in more detail, in chapter 2. Other methods exist for reaching consensus amongst a group of experts including the Nominal Group Technique (NGT) and the consensus development conference(648). More recently, Q methodology has been increasingly used in healthcare research(649). Each method has its own benefits, but all are subject to influences which may affect the outcome. These include: the way the task is set; the selection of participants; the selection and presentation of information; the structure of the interaction; and the method for synthesising judgements(648). In each case, when devising our Delphi
process, efforts have been made to follow best practice. In setting the task, we used an open round to reduce bias in the choice of cues, maintained the focus on creating a definition and, by combining the results of our open round with our literature review provided comprehensive choices for the following rounds. In selecting participants, we strove for a diverse group of professionals and recruited an adequate number. Above 12 participants, there appears to be diminishing returns in improvements in reliability (648). A limitation of our study was that the participants were skewed towards one country (Ireland). Also, we relied on implicit understanding of the term “expert”. These limitations should be addressed in future studies. In selecting and presenting information, the facilitator made efforts to include views from both sides of each particular argument when choosing narrative feedback to provide to participants between rounds. In addition, participants were provided with numerical feedback. The combination of both types of feedback has been shown to improve reliability (422, 423, 648). In structuring the interaction, a formal method (Delphi) was chosen, as more effective than informal methods (648). The number of rounds was set at 3. More rounds may lead to participant attrition, without necessarily increasing accuracy. A priori, as well as setting the number of rounds, we also defined consensus at 70%. Although these methods are widely accepted (352), other methods exist, such as continuing with voting until stability of responses is observed. It may be useful to consider alternative methods in future studies.

Definitions evolve over time, in line with scientific understanding, clinical considerations, and societal norms. In the development of future iterations, several factors may be considered. Recruitment of expert panellists is variable in the existing healthcare research which utilises the Delphi technique. Niederberger and Spranger have summarised the results of 12 systematic reviews of the use of the Delphi method in health sciences (650). They describe the various methods of selection of expert panellists. Third party recommendations, as would be seen in the snowballing recruitment technique that we employed, is recognised as a valid method of recruitment. However, an explicit definition of “expert”, may serve to increase confidence in the methodology of definition development. Methods to define experts more clearly include using institutional affiliation, number of years of experience, number of publications in a subject area, or academic title (650). Additionally, geographical aspects can play a role in composition of the expert panel (650). A more even spread of international participants may improve reliability and adoption of the definition. It may be useful for recruitment of each participant be performed by a member of the research team, rather than using a snowballing method. This would allow the researcher to exert more control over the international reach of the panel. In particular, it would be important to ensure participants from low and middle-income countries are included. Quotas on the number of panellists required from each region could be employed to guarantee that panellists are evenly distributed.
A broad multi-disciplinary panel was considered important in ensuring that the consensus-based definition of SNI was widely adopted, and participants were recruited from across the spectrum of healthcare professionals who care for these children. In the future, consideration should be given to including other professionals who care for children with SNI in the expert panel. Experts from the discipline of child psychiatry may be able to provide valuable insights into behavioural and emotional needs of children with SNI. This may add another level of depth to discussions about whether these elements should make up part of the definition, while continuing to utilise group consensus as the deciding factor. Expansion of the definition beyond the healthcare setting could allow for a more holistic description of the condition. For example, children with SNI have considerable interaction with the educational sector, and, often, several therapeutic interventions are provided to the child in school. Education professionals may, therefore, also provide additional insights into whether other concepts would be important to include in any future iterations of the definition. It is important that the definition remains user-friendly and attempts to expand the reach of the definition to other areas could make the definition unwieldy. Therefore, it is important that the intended use of the term remain central to any future processes to revise the definition.

A further limitation of this study was that we recruited only 2 parent representatives. Unfortunately, there were no parent representative organisations nationally which we could approach locally to recruit more parents. Our attempts to recruit parents through community disability services were unsuccessful as these organisations had no formal parent forums. We were also cognisant that we did not want to impose any additional time burden on the 2 parents that had volunteered. We envisage the creation of a parental forum from within our cohort, as we recognise this is a deficit and is important to any future work. The Irish Academy of Childhood Disability was launched September 2021 and may provide an important central point of contact between health professionals and the public. This may also facilitate recruitment of “experts by experience” to participate in future updates of the definition. Affiliation with international academies, such as the International Alliance of Academies of Childhood Disability (IAACD), could enrich public and patient involvement even further and ensure future research is relevant to those whose health it aims to improve.

To be truly useful, definitions must be widely adopted. To that end, we have published our literature review(353) and finalised definition of SNI(428) in international peer reviewed journals. We have also presented the definition, nationally and internationally. In the future, we envisage seeking endorsement of the definition by professional organisations. These organisations frequently have formal procedures which need to be completed prior to official endorsement, often including the appointment of content and methodological reviewers before consideration by their executive committee.
Formal endorsement would increase credibility and aid dissemination, further increasing adoption of the definition.

8.3 Cytokine responses in SNI

In chapter 4 we described altered cytokine responses in children with SNI. Although abnormalities in cytokines have been previously reported in children with CP(429), this study focusses on children with SNI, who have greater motor impairment, cognitive impairment, and medical complexity than an undifferentiated cohort of children with CP. In this study, we also significantly increase the numbers of children with neurological impairment in whom cytokine dysregulation is reported. Finally, we correlated cytokine responsiveness with several clinical markers including medication use, hospitalisation and CPCHILD™ score.

We have shown alterations in several pro- and anti-inflammatory cytokines in children with SNI. These results are supported by findings in other studies which have shown altered cytokine responsiveness in paediatric neurodevelopmental disorders. In neonates with NE, altered cytokines correlated with increased mortality, poorer neurodevelopmental outcome, and findings on MRI(253, 432). Sweetman et al demonstrated that elevated EPO in postnatal days 2-4 and lower VEGF in days 1-4 were significantly associated with the severity of NE in neonates exposed to perinatal asphyxia, and with death(253). EPO is a hypoxia-induced cytokine and has anti-apoptotic, anti-oxidative and anti-inflammatory actions(253). We showed EPO hyperresponsiveness of EPO to stimulation with LPS which may represent entrainment in our population. The cytokine abnormalities seen in neonates post-NE persist into later childhood, with these children shown to have raised baseline levels of GM-CSF, TNF-β, IL-2, IL-6 and IL-8, and LPS hyporesponsiveness in IL-10, VEGF, EPO and TNF-β(651). TNF-β was associated with poorer neurodevelopmental outcome. While the pattern of altered cytokines in this study is different from that seen in our research, it confirms that cytokine dysregulation persists following a neurological insult, raising the possibility that it is a contributory factor in later health related issues and the potential for use as a therapeutic target. Further evidence for cytokine dysregulation in children with neurodevelopmental disabilities is seen in its association with the development of CP(436). Furthermore, LPS hyporesponsiveness in several of cytokines, i.e. IL-1α, IL-1β, IL-2 and IL-6, is seen in school-age children with CP(429).

Cytokines were not different at baseline in the children with SNI compared to controls, but LPS hyperresponsiveness for EPO and LPS hyporesponsiveness for GM-CSF, and IL-6 was seen. There was a trend towards TNF-β and IL1-β hyporesponsiveness but this did not reach statistical significance. This is largely in keeping with previous research in which IL-6 and IL1-β were hyporesponsive to LPS in
children with CP(429) and TNF-β hyporesponsiveness was seen in children post-NE(127). In contrast, LPS hyporesponsiveness was seen for EPO in children post-NE, although in the latter cohort EPO was significantly higher than controls at baseline(127). Also, GM-CSF was found to be hyperresponsive to LPS in the post-NE group, a difference from our findings(323). These variations are likely to be secondary to differences in the populations studied. The post-NE group included children who had been diagnosed with NE in infancy, but excluded children who subsequently developed CP, whereas the children in our cohort had developed SNI as a result of a variety of aetiologies, which may have included NE. Nevertheless, children with neurological impairment have significant alterations in cytokine expression in response to LPS. The sample sizes to date have been small and larger studies are necessary to confirm the findings. Interestingly, higher serum and cerebrospinal fluid M-CSF, IL-6, and TNF-β concentrations, have been found to be associated with better neurological outcome in traumatic brain injury(652). The combination of these three cytokines has been termed M6T. M6T levels may be useful in predicting outcome in neurological injury but longitudinal studies with neonates and children will be required. In addition, in animal models, Li et al have demonstrated improved outcomes in neurological function, reduced volume of injured brain, and reduced neurone apoptosis in response to treatment with M6T(652). This may hold promise for children with SNI and the potential for randomised controlled trials could be explored in the future.

In chapter 4, we correlated cytokine responses with a variety of clinical outcome measures. We demonstrated positive associations between anti-epileptic drugs (AEDs), prophylactic antibiotics (most commonly macrolides), and percutaneous enteral feeding with cytokines which are traditionally considered anti-inflammatory, i.e., IL-10, IL-1ra, and EPO. In the cases of AEDs and prophylactic antibiotics, it is believed that their influence in increasing levels of anti-inflammatory cytokines is partially responsible for their effectiveness(472, 478, 479). Beneficial effects on systemic inflammatory states have been demonstrated in animal studies(481-486), but not yet in humans.

Effect of nutrition on cytokine regulation is less well described in the literature but our findings may suggest a possible link between enteral nutrition and inflammation. The role of nutrition in the modulation of inflammation and treatment of sepsis has prompted much interest in the literature. The gut represents a major interface between pathogens and the immune system and interruption of the functional integrity of the gut in critical illness may lead to translocation of bacteria, activation of inflammation, and provoke multi-organ dysfunction(653). In adult patients with sepsis, early enteral nutrition has been found to regulate T cell imbalance and suppress the IL-23/IL-17 axis, reducing the severity of sepsis(654). Enteral feeding in sepsis has been shown to alter levels of inflammatory cytokines such as IL-6(655). In neonates enteral feeding plays an important role in the
prevention of Necrotising Enterocolitis (NEC) and sepsis, and human milk is thought to exert a protective effect through its anti-TLR-4 properties(656). In children with sepsis, international guidelines for the treatment of septic shock and sepsis-related organ dysfunction showed preference for early enteral nutrition although evidence was inconclusive(657). Manipulation of enteral nutrition with supplements has garnered increasing interest in recent years. Omega-3 supplementation has been shown to reduce CRP, ESR, platelet count, white cell count and IL-6 in a prospective, double-blind, randomised control trial (n=120) in children with sepsis in the paediatric intensive care unit and also leads to shorter PICU admissions(658). The link between adequate nutritional intake and anthropometric measures in children with SNI and infection-related outcomes such as PICU admissions and mortality, requires further study. The potential to optimise nutrition to regulate inflammation, perhaps including supplements such as Omega-3 may hold promise as a therapeutic strategy in the future. The role of the microbiome in modulating the immune system and inflammatory response has been described in the literature(659, 660). Children with CP have been shown to have a different microbiota compared to healthy controls(661) and, in children with CP, those fed a liquid diet had a different microbiota with more gastro-intestinal symptoms than children those fed a normal diet(662). Blended/blenderized diets have garnered increasing interest in recent years and they are perceived as beneficial by parents, with improved gastrointestinal symptoms and overall wellbeing(663, 664). There is also evidence to suggest that a blended diet can lead to a more diverse microbiome in medically complex children(665). Therefore, considering the known links between neurodisability and microbiota, the microbiota and the immune system, it would be interesting to explore whether type of feeding in children with SNI has effects on cytokine expression. In particular, given parental preference for blended diets, it may be useful to examine the effects of this type of diet on inflammation. The benefit of deepening our knowledge in this area lies in the potential to modulate inflammation through manipulation of the microbiome, an area in which there is substantial interest and ongoing research(666-669).

Finally, we demonstrated an association between lower IL-18 responsiveness and number of respiratory tract infections. IL-18 is considered predominantly pro-inflammatory and stimulates production of reactive oxygen species as well as activating CD8+ T cells. The association we have demonstrated may be secondary to trained immunity or, conversely, reduced IL-18 responsiveness may contribute to the development of recurrent respiratory tract infections. IL-18 has potential to be useful clinically. Firstly, as a biomarker, IL-18 has been shown to be lower in patients with severe acute respiratory syndrome (SARS), and may be used to assess efficacy of treatment(670). Children with asthma show a reduced response of IL-18 to *Mycoplasma pneumoniae*, and more severe disease than non-asthmatic children(671). Reduced IL-18 may be useful as a marker of
more severe respiratory infection in children with SNI but larger studies, involving more children and a wider variety of pathogens would be required to determine the validity of using this as a predictive marker. IL-18 levels may be useful in personalising treatment decisions for children with acute respiratory tract infections. Oishi et al propose the use of steroid therapy in children with *M. pneumoniae* and IL-18 levels greater than 1000pg/ml(672). There is much interest in exploiting IL-18 as a potential therapeutic target, although most of the literature to date has focussed on IL-18 blockade in conditions such as sepsis, cancer, and autoimmune conditions(470, 673-678). However, IL-18 also has a protective effect(679) and administration of exogenous IL-18 has been shown to reduce mortality in certain situations, such as infection with *Toxoplasma gondii*(680).

Further research on age-related, pathogen-related, and situation-related effects of IL-18 are required and may lead to tailored therapy in the future.

### 8.4 Immune function in SNI

In chapter 5 we described proportions and function of lymphocytes and granulocytes using flow cytometry. We then described expression of genes associated with the NLRP-3 inflammasome. There is a relative lack of research on immune function in children with neurological impairment. However, several studies have suggested alterations in a variety of leukocyte proportions and functionality(126, 495-498). Also, as seen in chapter 4, and in previously published research(429), children with CP have cytokine dysregulation. It is also known that children with significant neurodisability are at a higher risk of infection related morbidity and mortality(52, 261, 492-494). Although this risk is multi-factorial, it is plausible that a significant role is played by immune dysfunction. The value in understanding the role of the immune system and inflammasome in this population lies in the potential to target them for therapeutic benefit.

We demonstrated a lower proportion of total T cells, CD8+ T cells, and monocytes, at baseline, in children with SNI compared to controls. T cells, especially cytotoxic CD8+ T cells are important in the defence against viruses and intracellular bacteria, while monocytes are vital in the early immune response to tissue injury and bridge the gap between the innate and adaptive immune systems. The clinical importance of reduced proportions of these cells in the population of children with SNI is not known, although the possibility of such is suggested by several, mostly adult, studies which demonstrate increased morbidity associated with lower T cell and monocyte proportions(497, 502, 503, 524-529). The reduced numbers of T cells and monocytes as we have reported is subclinical and many of the cell line proportions that we have discussed are not reported in routine clinical practice. It would be interesting to correlate these sub-clinical reductions in certain cell lines with frequency of respiratory tract infections, sepsis, hospital admissions and infection-related mortality. Future studies may focus on the effectiveness of a lower
threshold for prescription of prophylactic antibiotics, or of extended vaccine coverage in children with SNI.

We demonstrated relative hyporesponsiveness in neutrophil CD66b expression in children with SNI following exposure to LPS. CD66b is associated with neutrophil aggregation, adhesion, and migration. In adults, reduced activation of neutrophils is associated with higher mortality in sepsis(517). Paediatric studies are required to evaluate whether CD66b hyporesponsiveness translates to poorer outcomes in sepsis for this age-group.

We also described monocytic TLR-4 hyperresponsiveness to LPS in children with SNI. In particular, TLR-4 expression was noted to be higher in the classical monocytes, which are strongly pro-inflammatory. This may indicate a pro-inflammatory state, as suggested by previous research in children with NE and CP(127, 429, 681-683). TLR-4 over-expression is associated with abnormal neuroimaging in preterm neonates(682), neuroinflammation, and neurodegenerative diseases(684). Inflammation may constitute a mechanism of tertiary brain damage in children with CP(538). Thus, there is considerable interest in attenuating TLR-4 as a method of improving neurological outcome and modifying multi-organ dysfunction(538, 682, 684).

Finally, in chapter 5, we demonstrated that in children with SNI, a rise in NLRP3 or IL-1β gene expression was not exhibited in response to LPS, a finding which contrasted with the control population. This may represent a reduction in the innate inflammatory response and indicate a relative immunosuppression(430). Gurung et al demonstrated that prolonged exposure to LPS (>12 hours) dampens NLRP3 activation through IL-10 mediated regulatory mechanisms(685). In our population, the higher infection related morbidity seen in children with significant neurodisability is responsible for repeated stimulation of the innate immune system which may be leading to impaired inflammasome expression. The reverse may also be true, in that impaired inflammasome expression may be contributing to infection-related morbidity in these children. It is also possible that the altered immunological status of these children begins in utero. Research by Xu et al shows the altered cytokine responses (IFN-γ and IL-4) of children conceived by assistive reproductive technology indicating that antenatal triggers may be responsible for long term immune dysregulation(686).

In contrast to our findings, in patients with NE, an increased activation of the NLRP3 inflammasome is seen in the neonatal period which persists into childhood(430). Further study of the NLRP3 inflammasome, which has been implicated in numerous disease states, may serve to confirm our findings, and may provoke interest in its exploitation as a therapeutic target in modifying the inflammatory/immune response. Much of the existing literature focuses on NLRP3 inhibition as a method of reducing inflammation and improving outcomes in several disorders, including traumatic brain
injury(687, 688). However, evidence of potential benefit of enhancing NLRP3 activation is suggested by literature regarding patients following trauma who often exhibit immunosuppression(689). The NLRP3 inflammasome has been shown to be hyporesponsive in trauma patients, but transfection of monocytes from those patients with NLRP3-encoding plasmids recovered functionality, suggesting potential of the system as a target for treating immune suppression(690).

Although the numbers of children included in our study are small, it has contributed to the limited amount of literature published in the area. The alterations in leukocyte proportions and function, and inflammasome expression, may partially explain the increased infection related morbidity and mortality in children with SNI. Future research may focus on whether the markers of leukocyte activation, such as TLR-4 and CD66b, which we have shown to be altered in our population, or NLRP-3 inflammasome activation, provide useful biomarkers which predict outcomes such as recurrent infections, severity of illness, length of hospitalisation, and sepsis-related mortality in this cohort of children. Considering the increased infection-related mortality in children with SNI, early indicators of infection may allow early intervention to reduce hospital admissions and premature death. Information on immune dysfunction in the clinical setting may provide a method of deciding which patients are at higher risk of overwhelming infection, and thus allow the clinician to introduce prophylactic therapy such as antibiotics or vaccination.

8.5 Multi-organ dysfunction in SNI

We divided chapter 6 into 3 related short reports, describing various aspects of multi-organ dysfunction in children with SNI. We focussed on the renal and cardiovascular systems initially, before examining MOD in children with SNI more broadly and proposing a preliminary MOD scoring tool.

We demonstrated that children with SNI have significantly lower creatinine levels than controls. Creatinine is a biochemical marker frequently used to monitor renal function in the clinical setting. Children with SNI have reduced mobility with lower muscle mass, explaining the lower baseline creatinine levels(544). This presents difficulty in accurate monitoring of renal function in those with SNI, compounded by the fact that creatinine does not rise in the early stages of renal disease(543). Thus, creatinine levels may not be a reliable marker of renal dysfunction in children with SNI.

Cystatin C was used as a muscle-mass independent marker of renal function, and we demonstrated that children with SNI did not have higher levels than controls. This provides some reassurance that subtle renal disfunction is not being inadvertently missed. However, it is recommended that glomerular filtration rate (GFR) be used for monitoring renal function, rather than monitoring of creatinine or cystatin C levels alone(691). Accurate measurement of GFR is cumbersome, so, in practice estimated GFR (eGFR) is
used. eGFR is calculated based on creatinine, and/or Cystatin C, adjusted for body composition, which is estimated using height, weight and/or body surface area. These estimations do not take account of the different body compositions of children with neurodevelopmental disabilities such as CP who have higher fat mass and lower fat free mass for a given BMI, highlighting the difficulties in predicting body composition using traditional methods(692). Nor do they account for inaccuracies in measurement of height and weight, which can occur because of the inherent difficulties in taking these measurements in children who may have reduced mobility, contractures and several pieces of medical technology attached. Equations that calculate eGFR without height, use average creatinine for age in their calculations, which would be unreliable in children with neurodisability for the previously mentioned reasons. It would be useful, in future, to develop a method of calculating eGFR in children with SNI, which would allow for monitoring of renal function. Development of a new eGFR equation for children with neurodisability would be a complex process with a requirement for large numbers of participants from diverse backgrounds. In the adult population efforts have been made to improve estimations of GFR in cases where it is known that traditional estimations are inaccurate, such as in younger patients with diabetes or in transplant recipients(693). The latter study may provide a useful framework for the development of such an equation in children. Accurate monitoring of renal function would allow for earlier intervention with preventative measures, such as avoidance of nephrotoxic drugs, should initial signs of renal impairment be found.

We showed that children with SNI do not have significant differences in cardiac biomarkers, compared to controls. Both high-sensitivity Troponin T (HS Troponin T) and N-terminal prohormone b-type natriuretic peptide (NT-proBNP) are sensitive and specific markers of cardiac myocyte dysfunction(555, 694). They have been used in monitoring of children with various chronic conditions, including neurological disorders(144, 146, 558, 559, 694-696). It is reassuring that children with SNI do not show significant increases in these markers. Efforts to correlate biochemical markers with echocardiographic measures of function were hampered by the COVID-19 pandemic and the subsequent cyber-attack on the Irish health service. In future work, we will focus on detailed echocardiographic measurements of cardiac function in children with SNI and comparing them with age and sex-matched controls. It has been shown in other populations of children with chronic diseases that speckle tracking and tissue doppler are able to detect subtle ventricular dysfunction which is not detected by conventional echocardiography(571, 573, 697-699). Signs of subtle cardiac dysfunction in children with SNI may facilitate closer monitoring of these children with the possibility of intervening earlier to modify cardiometabolic risk factors(566, 567, 700), such as hyperlipidaemia and blood pressure, if more significant abnormalities are found.
We quantified multi-organ dysfunction in the children with SNI who participated in our study. We demonstrated the involvement of several systems in these children and showed the level of exposure to polypharmacy that these children experience. We proposed a preliminary scoring system for MOD in SNI which may be useful in communication, tracking progress over time, predicting outcomes, and as a tool in research. The Disabilities Complexity Scale, developed by Horridge et al.(577, 578), may help to provide a framework as to how to develop the SNI MOD score further although the former focuses on needs and complexity as opposed to the narrower focus of our preliminary MOD score. Work is ongoing, within CHI, in collaboration with international partners, on developing a common data set for children with SNI. The results of that process will help to refine the score further as those items which are chosen to be essential in the common data set could be incorporated into the SNI MOD score to increase its utility in future research. It may be useful to gain further insights from several stakeholders through a priority setting process, in collaboration with the James Lind Alliance (http://www.jla.nihr.ac.uk/about-the-james-lind-alliance). The James Lind Alliance aims to ensure public and patient involvement in research by allowing patients, carers and clinicians to come together to determine what are the most important research questions which need to be answered for a particular group. This has previously been performed for children with neurodisability in Britain(701). A process such as this would ensure that the outcomes of most interest to patients would be captured in a MOD scoring tool for children with SNI. Following refinement of the score, a process of validation will be required, and its utility proven in practice, such as has been done with the CPCHILD™ score(362, 613).

8.6 Family wellbeing in SNI

It is known that caring for children with chronic healthcare conditions has significant effects on the family(617). This is particularly true for children with chronic neurological disorders(2). Family relationships can become strained, with difficulties in communication, disruption to normal routines, the medicalisation of the home, and worries about the future(599, 611, 612, 702).

Little research exists which explores the distinctive needs of families of children with SNI. The experiences of each family are unique, and, even within the same family, there is evidence to suggest that each family member may have different perceptions and concerns about their situation(341). We sought to expand our understanding of family life for those caring for a child with SNI, with a particular focus on the siblings. Deepening our knowledge in this area may assist healthcare professionals in providing holistic care to the child with SNI, which is inseparable from the wellbeing of their family.

We reported the significant family impact of caring for a child with SNI, as reported by their parents. Parental quality of life (QoL) was lower than controls, with their physical
and social functioning noted to be particularly affected. This is in keeping with other research in the area and it would appear that greater levels of motor impairment may have a greater impact on parental QoL (596, 614).

Communication, with healthcare professionals and others, is deficient, and it is an area which could be improved with relatively little impact on scant resources. Parents reported issues with communication, but this was also perceived to be an issue in our focus group with teenage siblings. They reported the frustration of their parents’ voices not being heard. They also spoke about their perception that the medical management of their brother or sister was sub-optimal and discussed episodes in their care which they found frightening. Future research may focus on the most helpful strategies for communicating with families regarding their child’s healthcare, but it would be important to remember the impact that communication has on siblings as well as their parents. An integrative review of healthy siblings of children with cancer found that healthcare professionals should include siblings in assessments of family unit adaptation and provide interventions to promote their psychosocial wellbeing (642). It is most likely that parents will be the ones to communicate information regarding the health of their brother or sister. A framework has been developed to assist parents communicate with these healthy siblings (612). It would be interesting to explore the value of providing this framework to parents as an intervention to improve communication with siblings.

The parents’ concerns regarding others’ perception of their child with SNI is a wider societal issue. Siblings also expressed a hesitancy to discuss their brother’s or sister’s disability with those outside their family or circle of friends. Views and presentation of disability in the media and cultural values are just some of the issues which need to be considered alongside the findings of our research. These issues are likely to be difficult to tackle and slow to change.

Parents of children with SNI report significant worries both about their child’s medical condition and its effects on other members of the family. Interestingly, although parents worry about the impact of their child’s disability on other children in the family, we found no statistically significant difference in the QoL of siblings compared to controls. In our focus groups, the young people who participated appeared to appreciate the stress under which their parents operated. However, they did not report any negative emotions towards their parents or sibling, but, instead, described a sense of acceptance. In fact, one of the young people who participated explicitly stated that his sister’s SNI did not impact him much but had a great impact on his parents. They also described feeling more resilient, with a better ability to cope with adversity. It may be interesting, in future studies, to examine parental perception of the impact of their affected child’s condition on healthy siblings, and to compare these perceptions with those of their children.
Parents of children with SNI reported poorer family relationships, which may reflect the levels of stress that these families experience. The relationships which are perceived to be affected most are not explored further in the PedsQL™ FIM. Again, it would be interesting to compare parental experiences with those of their siblings because the teenagers who participated in our focus group reported largely positive experiences of parental support.

Sibling QoL as measured by the PedsQL™ was not significantly different from controls. This may reflect the small effect sizes that have been reported in other sibling studies. However, when viewed in the context of the wealth of information which became evident during the focus group, it is perhaps more likely that this reflects the relatively narrow view of QoL which is represented in questionnaires. The reality of living in the family of a child with SNI is, of course, exceptionally complex, and multi-faceted, with a myriad of external factors influencing perceptions.

Another element of complexity is added when one considers family composition e.g., single parent families, blended families etc. We did not gather such data in our study so cannot comment on whether this had any impact on our results. Future research including participants from a variety of family types may shed further light on whether this impacts on wellbeing. It may also be useful to expand this research to the wider family. For example, grandparents, who not only experience upset regarding their grandchild’s diagnosis, but must also witness the emotional distress of their own child as they deal with a life-changing situation(703).

In chapter 7, we discussed the predominance of responses from mothers, with little engagement from fathers. It seems that the experience of fathers differs from that of mothers(341) but there is a paucity of research in the area. A recent systematic review identified 32 papers from the period 1997-2019 which examined the experience of fathers solely, and these papers had a bias towards the oncology setting(607). Multiple factors are probably at play, influencing paternal participation, including work commitments, perceived societal norms and difficulty contacting fathers, amongst others(607, 647, 704). One must also consider that professionals may have a biased view of the role of fathers and may have poor awareness of their emotional needs(607). Nicholas et al have recently published a paper describing the barriers to fathers’ participation in research and providing useful strategies to improve their engagement(704).

The participants in our focus group were all aged between 12 and 14 years. Further research exploring the experiences of younger siblings of children with SNI will contribute further to the area, as younger children will have distinctly differing needs to adolescents. In addition, longitudinal, qualitative research studies would be useful to assess how siblings’ experiences evolve over time and would provide us with a deeper understanding of their needs.
8.7 Conclusions and Future Directions

Children with SNI are a unique and medically fragile group of children. We set out to assess variability in the use of the term SNI, place it in context of other definitions for children with chronic conditions, and establish the value of the term. We then described the process by which we created a consensus-based definition of SNI. Dissemination of the newly created definition continues. Future work will focus on attaining professional body accreditation for the term, which will augment dissemination further. In the medium term, the definition may be further refined, with consideration given to the opinions of a wider expert group. In particular, inclusion of other professions, augmentation of patient and public involvement, and expanding the international reach of the process may be beneficial. It may also be useful to monitor whether the use of the definition increases in the literature and whether, as a result, this leads to improved consistency in research in the area.

Studies regarding inflammation and immune function in children with neurological impairment are still in their infancy, and this is especially true for those with SNI. The immune dysregulation that we have demonstrated in this research adds to the existing literature in the area. There is growing evidence that children with CP and SNI have persistent altered inflammatory responses. The clinical implications of these findings remain uncertain, but the possibility that these alterations lead to tertiary neurological injury and impaired ability to respond to infection, warrant further large longitudinal studies, not least because there is significant potential to improve outcomes through immunomodulation.

Immune dysregulation is just one of the multi-system effects of SNI. We have demonstrated that SNI can be considered as a multi-organ disorder. We reported that biochemical markers of renal and cardiac function are not different in children with SNI compared to controls. However, challenges remain in assessing for dysfunction in either system, especially abnormalities which may be subtle. Future work could focus on developing accurate estimated glomerular filtration rate (eGFR) calculators in children with neurodisability. Detailed, longitudinal, echocardiographic assessment of cardiac function in children with SNI, and correlation with biochemical markers would be useful to further our knowledge of cardiac function in children with SNI over time. Children with SNI have involvement of several organ systems and are exposed to significant polypharmacy. We proposed a preliminary MOD score for children with SNI, which may be useful in quantifying multi-system involvement in these children, tracking natural history, and predicting outcomes such as mortality. Further refinement of the score will be required, with input from all relevant stakeholders, including public and patient involvement. Work is ongoing within our group, in conjunction with international collaborators, to develop core
outcome sets for children with SNI. The conclusions of that work will contribute to the final version of the SNI MOD scoring tool, which will then need to be validated in further studies.

We demonstrated the significant impact of caring for a child with SNI on the family, with almost all aspects of life affected. We reported that well siblings experience significant changes to their life as a result of having a brother or sister with SNI, although their experiences may be too complex to capture using existing objective tools, such as the PedsQL™. We highlighted the lack of research in fathers of children with SNI. Future work will focus on expanding our knowledge of the effects on siblings, perhaps using different methodology and including younger siblings. Through collaboration with others, we hope to increase the focus on the paternal experience within the family.

In conclusion, there is a growing recognition of the importance of research into the medical and psychosocial health of this vulnerable cohort of children with SNI. There is still much to learn about the complex and interconnected nature of immune dysregulation, persistent inflammation, and multi-organ dysfunction. The consensus definition of SNI provides the opportunity to study this group as a distinct cohort, so we may begin to understand symptom burden, healthcare utilisation, and important factors influencing good health outcomes as well as contributing to increased morbidity and mortality. This work will support clinicians in providing evidence-based information to families as we work together to make the best decisions for their child.

An understanding of the family dynamics and wellbeing of those surrounding the child with SNI is just as important and complex as the understanding of medical care. Further work to understand this will inform ways in which families can be supported and empowered to live their best lives.

This work has developed a basis for a holistic approach to research and clinical care for children and young people with SNI. Continued work in this area, with input from those affected, is needed to ensure the best outcomes for these children and their families are achieved.
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Appendices
Appendix i – Ethical approval letters and amendments

Dr John Allen
Lecturer Registrar
Trinity College Dublin
Trinity Centre for Health Sciences
Tallaght University Hospital
Dublin 24

14th September 2018

REF: SERENITY : Severe Neurological Impairment and children with medical Complexity

REC Reference: 2018-09 Chairman’s Action (7)
(Please quote reference on all correspondence)

Dear Dr Allen

The REC is in receipt of your recent request to SJH/TUH Research Ethics Committee in which you queried ethical approval for the above named study.

The Chairman, Prof. Richard Dean, on behalf of the Research Ethics Committee, has reviewed your correspondence and granted ethical approval for this study.

Yours sincerely,

[Signature]

Secretary
SJH/TUH Research Ethics Committee

The SJH/TUH Joint Research and Ethics Committee operates in compliance with and is constituted in accordance with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004 & ICH GCP guidelines.
Dr John Allen,
Tallaght University Hospital,
Tallaght,
Dublin 24

17th April 2019

Re: SERENITY: SEveRF Neurological Impairment and Children with medical complexity

REC Reference: 2019-04 List 13 (14)
Previous REC Reference: 2018-09 Chairman’s Action (7)
(Please quote reference on all correspondence)

EudraCT Number:

Date of Valid Submission to REC: 27.03.2019
Date of Ethical Review: 12.04.2019
R&I application Number: N/A

Dear Dr Allen,

Thank you for your correspondence in which you submitted an amendment for the above named study.

The Chairman has reviewed the documentation you submitted and has given approval for this study to proceed. The following comments were made:

- Please add the site to the PILs
- Please add contact details for Prof Molloy
- The data controller is TUH
- Please add a section outlining what information will be collected.

The following documents were reviewed:

- Non-clinical amendment form, dated 26.03.2019
- PILs x3, version 1, dated 07.02.2019
- Information to legally accepted representative of participant, version 1.0, dated 04.03.2019

Applicants must submit an annual report for ongoing projects and an end of project report upon completion of the study. It is the responsibility of the researcher/research team to ensure all aspects of the study are executed in compliance with the General Data Protection regulation (GDPR), Health Research Regulations and the Data Protection Act 2018. Additionally, please note for documents submitted for GDPR purposes that the REC and the Chair are not confirming that you’re documents are GDPR compliant, they are approving the document from an ethical perspective.

Yours sincerely,

REC Officer – Dr Sadhbh O’Neill - SJH/TUH Research Ethics Committee
Dr John Allen,
Tallaght University Hospital,
Tallaght,
Dublin 24

13th February 2020

REF: SERENITY: Severe Neurological Impairment and Children with Medical Complexity

REC: 2020-02 List 5 – Amendment (15)
(Please quote reference on all correspondence)

Date of Valid Submission to REC: 04.02.2020
Date of Ethical Review: 07.02.2020

Dear Dr Allen,

The Chairman, Prof. Richard Deane, on behalf of the Research Ethics Committee, has reviewed the amendment you submitted to the SJH/TUH JREC for the above named study and has given FULL approval for this amendment. Please outline the pathway should a patient indicate distress when completing the questionnaire.

The following documents were reviewed:

- Amendment Request Form, dated 03.02.2020
- PedsQL (Child/Teen 13-18yrs, 8-12yrs, 5-7yrs, 2-4yrs), V4, dated 11.05.2018
- PedsQL (Parent 13-18yrs, 8-12yrs, 5-7yrs), V4, dated 11.05.2018
- GP Questionnaire

Please note that ethical approval for this study is only active under the following conditions:

1. Applicants must submit an annual report for ongoing projects.
2. Applicants must submit an end of study declaration/end of study report upon completion of the study.
3. All adverse events must be reported to the JREC.
4. All changes (minor and substantial) to documentation/study must be submitted to the JREC using the amendment request form and the changes must be tracked/highlighted clearly. Approval from the JREC is required before implementation of the changes.

It is the responsibility of the researcher/research team to ensure all aspects of the study are executed in compliance with the General Data Protection regulation (GDPR), Health Research Regulations and the Data Protection Act 2018.

Yours sincerely,

REC Officer – Dr Sadhbh O’Neill

The SJH/TUH Joint Research and Ethics Committee operates in compliance with and is constituted in accordance with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004 & ICH GCP guidelines.
Dear Dr Allen,

The Chairman, Prof Richard Deane, on behalf of the Research Ethics Committee, has reviewed the amendment you submitted to the SJH/TUH JREC for the above named study and has given FULL approval for this study to proceed.

The following documents were reviewed:
- Non-Clinical Amendment Form, dated 02.03.2020
- Child’s Sleep Habits Questionnaire
- Sleep Disturbance Scale for Children
- Participant Information Leaflet, V2, dated 27.02.2020
- Patient Consent Form, dated 27.02.2020

Please note that ethical approval for this study is only active under the following conditions:
1. Applicants must submit an annual report for ongoing projects.
2. Applicants must submit an end of study declaration/end of study report upon completion of the study.
3. All adverse events must be reported to the JREC.
4. All changes (minor and substantial) to documentation/study must be submitted to the JREC using the amendment request form and the changes must be tracked/highlighted clearly. Approval from the JREC is required before implementation of the changes.

It is the responsibility of the researcher/research team to ensure all aspects of the study are executed in compliance with the General Data Protection regulation (GDPR), Health Research Regulations and the Data Protection Act 2018.

Yours sincerely,

REC Officer – Dr Sadhbh O’Neill
Dr John Allen,
CHI at Tallaght,
Tallaght,
Dublin 24

21st April 2021

REF: SERENITY: Severe Neurological Impairment and Children with Medical Complexity

REC: 2021-04 Chairman’s Action (29)
(Please quote reference on all correspondence)

Date of Valid Submission to REC: 21.12.2020
Date of Ethical Review: Feb 2021

Dear Dr Allen,

The Chairman, Prof. Richard Deane, on behalf of the Research Ethics Committee, reviewed correspondence which you submitted to the SJH/TUH JREC and has given FULL APPROVAL for the above named study to proceed.

The following documents were reviewed:

- Standard Application Form
- Response to Ethics Committee
- Questionnaire

Please note that ethical approval for this study is only active under the following conditions:

1. Applicants must submit an annual report for ongoing projects.
2. Applicants must submit an end of study declaration/end of study report upon completion of the study.
3. All adverse events must be reported to the JREC.
4. All changes (minor and substantial) to documentation/study must be submitted to the JREC using the amendment request form and the changes must be tracked/highlighted clearly. Approval from the JREC is required before implementation of the changes.

It is the responsibility of the researcher/research team to ensure all aspects of the study are executed in compliance with the General Data Protection regulation (GDPR), Health Research Regulations and the Data Protection Act 2018.

Yours sincerely,

REC Officer – Ms. Chita Murray
SJH/TUH Research Ethics Committee

The SJH/TUH Joint Research and Ethics Committee operates in compliance with and is constituted in accordance with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004 & ICH GCP guidelines.
ETHICS (MEDICAL RESEARCH) COMMITTEE OFFICE
Tel: + 353 (01) 409 6307/6243

Dr John Allen
Registrar lecturer in Paediatrics
Paediatrics
Trinity Centre for Health Sciences
Tallaght University Hospital
Dublin 24

10th March 2021

REC Ref: GEN/902/21

SERENITY: SEveRE Neurological Impairment and children with medical complexity
Principle Investigator: Prof Eleanor Molloy, Dr John Allen

Dear Dr Allen,

The Ethics Committee at a meeting which took place on the 9th March 2021, reviewed the above named project. The Ethics Committee would like to thank Prof Molloy and yourself for attending the meeting and taking their questions.

The Ethics Committee have recommended that your request to move the echocardiogram portion of your research study to CHI at Crumlin be APPROVED. It is also acknowledged that you will need access to patient records in CHI at Crumlin as part of this move.

One comment that the Ethics Committee raised involved the wording in the control PIL. It should be ensured that control participants or their parents are not under the impression that they could have a neurological impairment.

I wish you every success with the study and should you need any assistance in the future do not hesitate to contact the Research Ethics Office.

Yours sincerely,

__________________________________
Dr Eugene Dempsey
CHI Research Ethics Officer

Cc: Prof Eleanor Molloy, Professor of Paediatrics and Child Health, Trinity College Dublin
## Participant Information Leaflet

**Study title: SERENITY**

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<tr>
<td>Telephone number of principal investigator:</td>
<td>+353 1 4142671</td>
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<tr>
<td>Address of principal investigator:</td>
<td>Trinity Centre for Health Sciences, Tallaght University Hospital, Dublin.</td>
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<tr>
<td>Co-Principal investigator’s name:</td>
<td>Professor Denise McDonald</td>
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<tr>
<td>Co-Principal investigator’s title:</td>
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Version 3  Date: 05/10/2020  282
Study Site: Tallaght University Hospital

You are being invited to take part in a research study to be carried out at Trinity College Dublin by Dr. John Allen, under the supervision of Professor Eleanor Molloy and Professor Denise McDonald.

Before you decide whether you wish to take part, you should read the information provided below carefully. Take time to ask questions – don’t feel rushed and don’t feel under pressure to make a quick decision.

You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you. This process is known as ‘Informed Consent’.

You don’t have to take part in this study. If you decide not to take part, it won’t affect your future medical care.

You can change your mind about taking part in the study any time you like. Even if the study has started, you can still opt out. You don’t have to give us a reason. If you do opt out, rest assured it won’t affect the quality of treatment you get in the future.

Why is this study being done?

We believe this study has the possibility to make a serious impact on child health. In this study we aim to find out the different issues that children with neurological impairment and medical complexity face. As an example, think about children with Cerebral Palsy (CP) who commonly have issues with movement. They can also have problems with other parts of the body, for example, they can have more infections in the lungs and can have difficulty sleeping.

If we know the types of problems that children with neurological impairment and medical complexity have, then we can plan resources to improve their health. We plan to look at quality of life, family wellbeing, sleep and function of the immune system in children with complex medical needs.

Who is organising and funding this study?

This study is organised by Dr John Allen who is currently working towards a PhD with Trinity College Dublin. He is supervised by paediatric consultants, Prof. Denise McDonald and Prof. Eleanor Molloy.

Dr John Allen’s salary is currently paid by Trinity College Dublin in his role as a lecturer and his academic fees are paid by the National Children’s Research Centre. Dr John Allen is conducting a project called SERENITY; SEvERE Neurological Impairment and children with medical complexity.

Ethical approval has been obtained from Tallaght University Hospital Research Ethics Committee in 2018.
Why am I being asked to take part?

We are asking parents of children with neurological impairment and complex medical needs if they would like to participate in this research project. We want to understand more about your child’s quality of life, family wellbeing, sleep habits, infections and other medical complications, and we would value your input in this process. Your participation is completely voluntary.

What will happen to me if I agree to take part?

If you volunteer to participate, we would like to assess your child’s clinical examination and medical history when you present for routine clinic appointments that your child is attending under the care of Professor Denise McDonald. We will also use your child’s health care records for this purpose, this will involve looking at their file and any electronic records that exist, for example blood results and radiology reports.

If you volunteer to participate, we will ask you to complete some surveys which look at the quality of life of your child and sleep habits and medical complexity. If your child is having a sleep study performed, we would like to be able to access the data from this study.

If your child is having bloods taken when they attend clinic, we may ask to study your child’s blood to look at the body’s inflammatory reactions and immune system.

If your child is having an echocardiogram (heart scan), we would like to be able to access this data and take some extra measurements. This will not cause any extra discomfort but may take a little extra time. We may also ask for some children to voluntarily have a heart scan performed by one the cardiology doctors, even though it may not be required as part of their routine care. Please be assured that these scans are painless and do not involve any radiation exposure. Participation, as with all other parts of the study, is completely voluntary.

We may also like to contact you in the future to invite you to participate in a study of family wellbeing, but this will also be completely voluntary.

If you do not wish to participate your care will continue as normal. Your child does not have to take part in the study and you do not have to explain why to anyone. Your child’s treatment will in no way be affected if he/she refuses to take part in the study. There is also no problem if you wish to participate in one part of the study but not in another.

Will there be video and/or audio recordings?

There are no video or audio recordings being taken in this part of the study.
What are the benefits?

There are no direct benefits to taking part in this study. However, those who take part in this research will be contributing to improving the care of all children with neurological impairment and medical complexity into the future.

What are the risks?

We don’t expect any risks from taking part in the study. However, we will be accessing your child’s medical records and understand that some of this information may be sensitive. The main risk arising from this would be a data breach, but we will take every step possible to ensure that this does not occur. We have outlined all the steps we take to protect your data in the sections below.

We understand that your time is valuable and will strive to ensure that all the questionnaires are as brief as possible.

Is the study confidential?

All of the personal data that we gather will be treated confidentially. Only the researchers will have access to this data. Any personal information that we have will be pseudonymised, meaning it could not be linked to you or your child without other information which is stored elsewhere. Identifiable information will be stored on a password-protected computer in a locked office. Following completion of the research study the data will be kept as long as required by the Hospital policy. The data will then be destroyed according to this same policy.

Blood samples:
If a small sample of blood is taken, the blood will be stored in the laboratory in Tallaght University Hospital. It will subsequently be brought to Trinity Translation Medicine Institute, St James’ Hospital. The sample will be stored until completion of the project. It will be stored under the Research Patient number.

Results:
This study will take up to 5 years to complete, you will not be notified of any results specific to your child. The results of this research project will be published in medical journals and presented at medical conferences. No identifiable information will appear in publications or presentations. The publications will be available on the Trinity website. https://www.tcd.ie/medicine/research/researchers/MOLLOYEL

Data Protection

1. We will be using your personal information in our research to help us determine the issues that children with medical complexity face.
2. The legal basis for the processing of your data is that it is in the public interest and for scientific research which fall under Articles six and nine of the General Data Protection Regulations 2016 (GDPR).

3. Only Dr John Allen and his supervisors Prof Eleanor Molloy and Prof Denise McDonald will have access to your information.

4. Data will be stored until completion of the project and publication of results. We hope this will be within 5 years. After this it will be destroyed.

5. While every effort will be made to protect your data, there is always a small possibility of a data breach, which may mean that your personal details are accessed by a third party. If this were to occur, we would inform you as soon as we became aware of the breach.

6. You have the right to withdraw your consent at any time by contacting Dr John Allen by email at allenj2@tcd.ie

7. You have the right to lodge a complaint with the Data Protection Commissioner.

8. You have the right to request access to your data and a copy of it by emailing allenj2@tcd.ie

9. You have a right to restrict or object to processing of your data.

10. You have a right to have any inaccurate information about you corrected or deleted.
11. You have a right to have your personal data deleted, unless the results have already been published.

12. You have a right to have your data transferred to another data controller in a readable format.

13. There will be no automated decision-making, including profiling.

14. You have the right to object to automated processing if you wish.

15. If we would like to use your information for another purpose other than that stated above, we would need to inform you of that wish and ask for your consent to use your information for this other purpose.

16. We do not intend to transfer any data outside of the EU, but if that issue arose, we would need to inform you and ask for your consent.
Data will be held until completion of the project and until publication of results. We hope this will be within five years. During this time period data will remain stored in an encrypted locked computer in a locked room. Biological samples will be held in the Trinity College laboratory at the St James’ site, Trinity Translation Medicine Institute, St James’ Hospital.

We would like to obtain your permission for your child’s data/biological material to be used for the current research and that we could store the data/biological material for possible future uses in research in cerebral palsy/neurological impairment and children with medical complexity. This would likely be research undertaken by the Paediatric group in Trinity College Dublin. Any such research must remain in keeping with recognised ethical standards for scientific research. Participation is voluntary. No data will be shared with commercial entities. All data will be pseudo-anonymised.

If you wish to withdraw from partaking in future studies, please contact Dr John Allen via email or phone. Details of how to contact him are at the end of this document.

**Where can I get further information?**

If you have any further questions about the study or if you want to opt out of the study, you can rest assured it won't affect the quality of any treatment you may get in the future.

If you need any further information now or at any time in the future, please contact:

Dr John Allen  
Project: SEveRE Neurological Impairment and children with medical complexity  
Address: Trinity Centre for Health Sciences, Tallaght University Hospital  
Phone No: 01 896 3763  
Email: allenj2@tcd.ie

Thank you for taking the time to read this Information Sheet and for considering your child to taking part in this study. If you have any questions or concerns, you can contact the Research Staff.
## PATIENT CONSENT FORM

**Study title: SERENITY**

<table>
<thead>
<tr>
<th>Statement</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>I have read and understood the Information Leaflet about this research</td>
<td></td>
<td></td>
</tr>
<tr>
<td>project. The information has been fully explained to me and I have been</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>able to ask questions, all of which have been answered to my satisfaction.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I understand that I don’t have to take part in this study and that I can</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>opt out at any time. I understand that I don’t have to give a reason for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>opting out won’t affect my future medical care.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I am aware of the potential risks, benefits and alternatives of this</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>research study.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I give permission for researchers to look at my medical records to get</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>information. I have been assured that information about me will be kept</td>
<td></td>
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<tr>
<td>private and confidential.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have been given a copy of the Information Leaflet and this completed</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>consent form for my records.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I consent to take part in this research study having been fully informed</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>of the risks, benefits and alternatives.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I give informed explicit consent to have my data processed as part of this</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>research study.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I consent to be contacted by researchers as part of this research study.</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>I consent to having a small blood sample collected from my child.</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>I consent to my child having an echocardiogram (heart scan)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>I consent to be re-contacted by researchers about possible future research</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>related to the current study for which I may be eligible.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### STORAGE AND FUTURE USE OF INFORMATION

**RETENTION OF RESEARCH MATERIAL IN THE FUTURE [please choose one or more as you see fit]**

| OPTION 1: I give permission for material/data to be stored for possible    | Yes | No |
| future research related to the current study only if consent is obtained  |     |    |
| at the time of the future research and only if the research is approved by |     |    |
| a Research Ethics Committee.                                              |     |    |
To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.
CHILD ASSENT FORM SERENITY
12-16 years old

This form provides you important information about the research study you are being asked to participate in. Please read it carefully! When you are finished you should know what the research study is about, what you will be asked to do and what are the likely risks and benefits. If you agree to participate, you will be asked to sign this form. A copy of the form should be given to you.

SERENITY: SEveRE Neurological Impairment and children with medical complexITY

PURPOSE OF STUDY:
We are conducting a research study to see if there are differences in your blood compared to children with disabilities. A research study is a way for scientists to learn more about people. In order to do this, scientists need volunteers to participate in their research. You are being asked to be a healthy volunteer because bloods are already been taken from you.

RESEARCH PROCEDURES:
If you decide to participate, you will be asked to give us an extra blood sample when bloods are being taken. We won’t insert the needle separately from the bloods taken as routine.

RISKS AND/OR DISCOMFORTS:
You should know that there is a possibility the procedure may be slightly painful.

BENEFITS:
We do not know if this study will help you, personally. We may learn something that will help others someday.

ALTERNATIVES:
You have the option of not participating in the study.

PRIVACY AND CONFIDENTIALITY:
The information that is collected in this research study will be kept private and confidential. This means that we will do our best to not let anyone see or hear the information you give to us while you participate or after. We will protect your information by changing your name by a number (code).

COMPENSATION:
You will not receive any compensation for participating in the study.
RIGHT TO WITHDRAW:
It is your choice to take part in this study. You do not have to be in this research study if you do not want to. You can say yes now and change your mind later. No one will be mad at you. Even if your parents give permission for you to participate, you can still say no. If we think it is best for you not to be in the research study, we may take you out of the study.

CONTACT INFORMATION:
We are happy to answer any questions you have about the study now or later. If you want to contact the researchers, you may call

Prof Eleanor Molloy
Address: Paediatrics, Academic Centre, Tallaght Hospital, Trinity College, The University of Dublin, Ireland
Phone No: 01 896 3763
Email: eleanor.molloy@tcd.ie

PARTICIPANT CONSENT:
You can take your time in deciding if you want to participate. If you sign below it means you agree to participate. It also means that you have read this document, understand what it means and the researchers have answered all of your questions.

______________________________________________
Printed Name of Participant

______________________________________________
Name and signature of Person Obtaining Assent

______________
Date
CHILD ASSENT FORM SERENITY
8-12 years old

SERENITY: SEveRE Neurological Impairment and children with medical complexiTY
Principal investigator: Prof. Eleanor Molloy

You are being asked to decide if you want to be in this research study because bloods are already been taken from you.

If you agree to be part of the study, we will take an extra blood tube at the same time that bloods are being taken as part of your treatment. We will also collect some of your information, but do not worry it will be kept safe and secure.

If you have any questions you can ask about this study at any time.

You can stop being in the study at any time without anyone being mad at you. Your doctor will still take care of you.

If you want to be part of the study

_____________________________________________
Printed Name of Participant

______________________________________________
Name and signature of Person Obtaining Assent

______________
Date
<table>
<thead>
<tr>
<th>You are getting your blood checked today</th>
</tr>
</thead>
<tbody>
<tr>
<td>If it’s going ok... I can have an extra sample</td>
</tr>
<tr>
<td>It will help me with my work</td>
</tr>
<tr>
<td>This will help other children</td>
</tr>
</tbody>
</table>