SIGNEC U.K. and the First International Conference on Necrotising Enterocolitis

Speakers and chairs at the first SIGNEC U.K. International Conference. From left: Minesh Khashu, Tim Wolfs, Jörn-Hendrik Weitkamp, Cheryl Battersby, Simon Eaton, Kate Costeloe, Phillip Gordon, Jayanta Banerjee, Andy Ewer, Michele Upton, David Paul and Joanne Ferguson.

Introduction

The special interest group for necrotising enterocolitis (SIGNEC U.K.) was set up by Dr Minesh Khashu. Initially comprising healthcare professionals from the UK, the group now has international involvement and includes neonatologists, paediatricians, surgeons, dietitians, transfusion medicine specialists, epidemiologists, basic science researchers, nurses, trainees and other healthcare professionals with an interest in necrotising enterocolitis (NEC) and health improvement. The aim of SIGNEC U.K. is to facilitate knowledge sharing, networking and collaboration to optimise research and improvements in practice.

NEC is the most common gastrointestinal emergency occurring in neonates. Its aetiology and pathogenesis are not fully understood but current opinion suggests an acute inflammatory disease with a multifactorial aetiology. It is characterised by variable damage to the intestinal tract ranging from mucosal injury to full-thickness necrosis and perforation.

NEC represents a significant clinical problem, affecting close to 10% of infants who weigh less than 1,500g, with mortality rates of up to 50% depending on severity of illness[1-3]. It is also associated with significant long-term gastrointestinal and neurodevelopmental morbidity[3,4].

In the absence of a definite aetiology and pathogenesis, efforts at prevention, minimising risk and optimising early management have been difficult. It would be fair to say that this is one area of neonatology that has not seen much progress in the last few decades. As highlighted at the conference, NEC is the 'disease of the day' for the neonatal community.

The SIGNEC U.K. first international conference on NEC took place on 8-9 July 2013. One hundred consultant neonatologists, paediatricians, dietitians, advanced neonatal nurse practitioners, neonatal nurses, paediatric trainees, basic science researchers and parent representatives attended the two-day event, held at Chelsea Football Club in London. Dr Khashu welcomed delegates to the meeting and set the scene by introducing the important contribution of emerging research to the field of NEC. While the first day of the conference addressed key areas of laboratory research, the second day focused on clinical practice. Twelve experts from the UK, USA and the Netherlands (**TABLE 1**) presented their work, which received a great deal of interest from the audience and generated a lot of discussion; selected highlights from the talks are discussed overleaf. There was plenty of opportunity for networking and the event was accredited by the Royal College of Paediatrics and Child Health (RCPCH) for 8.5 continuing professional development (CPD) points.

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SIGNEC U.K. SPECIAL INTEREST GROUP NEC, U.K.

infant

DANONE BABY NUTRITION

This supplement is based on a two-day conference that was supported by an educational grant from Danone Baby Nutrition.

Laboratory research and emerging frontiers

Antenatal inflammation and the fetal gut

Dr Tim Wolfs from Maastricht University opened the presentations by speaking on his research into chorioamnionitis and the preterm gut. Chorioamnionitis – inflammation of the fetal membranes during pregnancy due to infection – is closely associated with preterm birth and is an independent risk factor for intestinal pathologies such as NEC[5].

Dr Wolfs' work into the mechanisms underlying the association between chorioamnionitis and NEC in a sheep model suggests that the cytokine interleukin-1 (IL-1) is, at least in part, responsible for the detrimental intestinal inflammatory response seen in chorioamnionitis. IL-1 appears to be the dominant cytokine mediating the inflammatory response in the gut and *in utero* exposure to IL-1 results in a damaged mucosal intestinal barrier and disturbed immune homeostasis, in particular diminished numbers of mucosal regulatory T cells. These immune-mediated *in utero* changes are known risk factors for NEC pathology, indicating that postnatal problems may have antenatal origins. This concept opens new avenues for prevention of NEC, which Dr Wolfs further discussed in his second presentation on Day 2. The therapeutic potential of an IL-1 receptor antagonist (IL-1RA) raises exciting possibilities and experimental evidence suggests that IL-1RA prevents the inflammation of the fetal gut and morphological injury associated with IL-1 mediated inflammation.

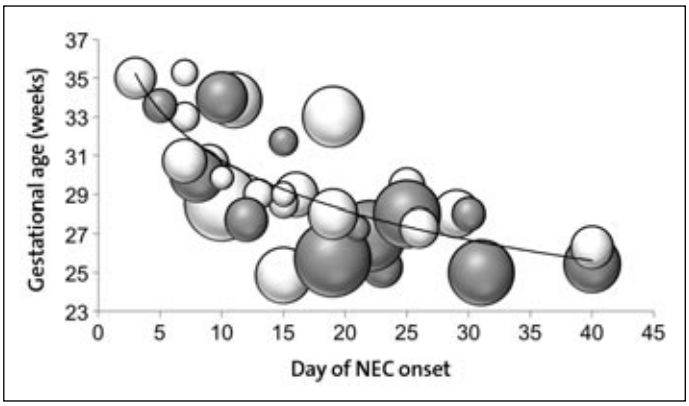

FIGURE 1 The timing of NEC onset tightly correlates with gestational age at birth.

Dr Wolfs briefly described how mortality in NEC animal models could be prevented by other immune modulating interventions such as stem cell therapy and the use of probiotics.

Effect of blood transfusion on gut perfusion in preterm infants

In his talk, Dr Jayanta Banerjee explained how gut perfusion could be measured using near-infrared spectroscopy (NIRS) and Doppler ultrasound scanning. Results from the current study at Homerton Hospital could lead to a better understanding of the impact of blood transfusion on gut perfusion in preterm infants and generate ideas for minimising risk.

Day 1: Laboratory research and emerging frontiers	
Dr Tim G.A.M. Wolfs *Head of the Laboratory of Paediatrics, Maastricht University, Netherlands*	Antenatal inflammation and the fetal gut
Dr Jayanta Banerjee *Clinical Research Fellow, Homerton University Hospital, London, UK*	Effect of blood transfusion on gut perfusion in preterm infants
Dr Andy Ewer *Consultant in Neonatal Medicine, Birmingham Women's Hospital, UK*	Volatile organic compounds and NEC
Dr Ingrid Renes *Danone Nutricia, Early Life Nutrition, Netherlands*	Research into gut development
Day 2: Conquering NEC: The difficulties and the way forward	
Joanne Ferguson *Patient and Public Engagement Representative, National Neonatal Clinical Reference Group, UK*	The traumatic journey
Professor Phillip V. Gordon *Associate Director of Research, Pediatrix Inc, Florida, USA*	NEC – the one disease that is many!
Dr Jörn-Hendrik Weitkamp *Assistant Professor of Paediatrics, Vanderbilt University, Nashville, USA*	Development of intestinal immune regulation and NEC
Dr Cheryl Battersby *Clinical Research Fellow, Imperial College London & Neonatal Data Analysis Unit, UK*	U.K. Neonatal Collaborative NEC study
Dr Simon Eaton *Senior Lecturer in Paediatric Surgery and Metabolic Biochemistry, UCL Institute of Child Health, London, UK*	Improving the outcomes from surgical NEC
Michele Upton *Innovation Lead, East of England Perinatal Network, UK*	A network approach to reducing NEC: the East of England experience
Dr Tim G.A.M. Wolfs *Head of the Laboratory of Paediatrics, Maastricht University, Netherlands*	Antenatal inflammation and the compromised fetal gut: what are the clinical consequences?
Professor David A. Paul *Director of Neonatal Research, Christiana Care Health System, Newark and Professor of Paediatrics, Thomas Jefferson Medical College, Philadelphia, USA*	Blood transfusion and NEC: causation or association?

TABLE 1 Programme of speakers.

Volatile organic compounds and NEC

Dr Andy Ewer highlighted the difficulties in early diagnosis of NEC and discussed how volatile organic compounds (VOCs), generated from bacterial breakdown of food in the gut, may offer a 'chemical fingerprint' in stool samples that may potentially aid early diagnosis of NEC. Pilot data from the colloquially named 'Pooh' study indicated that four specific esters are consistently absent up to four days prior to NEC onset and up to 12 days after. Dr Ewer explained the rationale behind the ongoing DOVE study (Diagnostic test of Organic Volatiles in necrotising Enterocolitis); a case-control study to compare VOC profiles in healthy infants and neonates with NEC. This may provide potential for cotside testing and early diagnosis of 'impending NEC' – an opportunity to minimise risk by modifying practice.

Research into gut development

Dr Ingrid Renes discussed the underlying causes of changes in gut structure and function in NEC. The small intestine of premature infants with NEC displays atrophy of the villi and death of epithelial cells, leading to a diminished absorptive capacity and a decrease in nutrient uptake. A contributing factor to this appears to be down-regulation of expression of epithelial-specific genes coding for nutrient digestion and transport. Additionally, in NEC, bacterial and cytokine-induced intestinal epithelial cell stress leads to ER (endoplasmic reticulum)-stress and the so-called unfolded protein response, ie a response aimed to resolve ER-stress. Prolonged ER-stress, which occurs in NEC, ultimately results in disruption of normal epithelial cell function and programmed cell death.

Conquering NEC: the difficulties and the way forward

The traumatic journey

In the first presentation on Day 2, Joanne Ferguson, a patient and public engagement (PPE) representative, described the traumatic journey for parents of a baby with NEC. Joanne highlighted that a better understanding of the impact on the mental health of parents is crucial. She also championed the need for research into the use and impact of online technology for information and support.

NEC – the one disease that is many!

In his talk, Professor Phillip Gordon discussed the reasons for NEC vulnerability, especially the biological basis of 'who, why and when'. He eloquently demonstrated how there is a window of vulnerability in that the timing of NEC onset tightly correlates with gestational age at birth; the younger the gestational age, the later the development of NEC (**FIGURE 1**), with the incidence of NEC peaking at 33-34 weeks' post-menstrual age. He reviewed the molecular and cellular basis for this, describing the role of Toll-like receptor signalling, macrophages, TGF-β2, the hyperinflammatory response by the innate immune system and regulatory T cell involvement. Recognising this high-risk window in which babies are likely to be at risk from various triggers of NEC, empowers clinicians to focus their approach to prevention and quality improvement programmes – strategies that SIGNEC U.K. believes are important for the future.

Professor Gordon went on to remind the audience that NEC is one of many acquired neonatal intestinal diseases (ANIDs). Spontaneous intestinal perforation (SIP) is a separate disease entity from NEC yet SIP can be a substantial contaminant within NEC databases. Establishing clean datasets is crucial; separating NEC from SIP and subsequently reducing NEC into subgroups (NEC reductionism) will improve identification and understanding of NEC (**FIGURE 2**). As his talk progressed, Professor Gordon discussed further aspects of NEC including antibiotic exposure and NEC, optimal rate of advancement of enteral feeds, and pathogen and non-pathogen triggers for NEC.

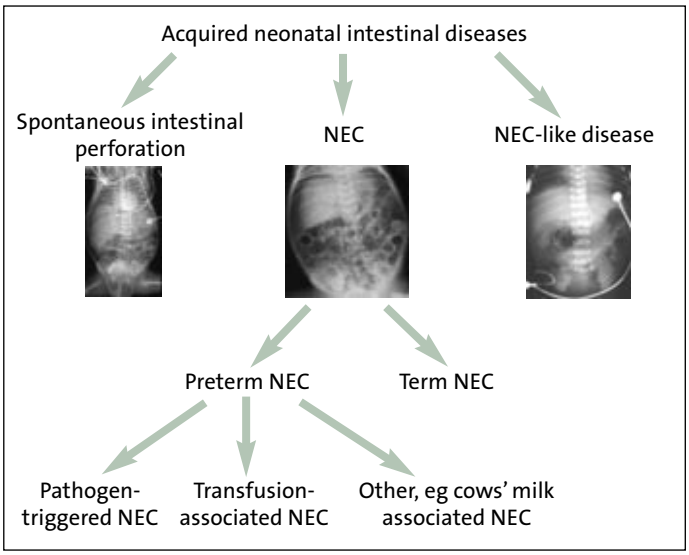

FIGURE 2 NEC reductionism – dividing NEC into reproducible subgroups.

The UK Neonatal Collaborative NEC study

Dr Cheryl Battersby highlighted the lack of a true definition for NEC and therefore the difficulty in objectively identifying cases. An unambiguous case-definition is crucial to determine incidence, for clinical research and to investigate management and outcomes.

There is no diagnostic test and, unless a baby undergoes surgery, it is difficult to know if they truly have NEC. The UK Neonatal Collaborative NEC study aims to establish a definition for NEC that is suitable for national and international surveillance, enabling determination of the population incidence of NEC in England. This should also allow identification of enteral-feed related factors that precede onset of NEC, in order to inform the design of future interventional randomised controlled trials (RCTs).

Improving the outcomes from surgical NEC

Surgical outcomes for NEC have not particularly improved over recent years. Dr Simon Eaton explained how there will always be some infants who require surgery and how the outcome from surgery depends upon the degree of intestinal involvement: mortality from focal disease is less than that from multifocal NEC, which is less than that from pan-intestinal NEC. He then went on to discuss RCTs for surgical interventions for NEC, in particular trials comparing peritoneal drainage vs laparotomy, and resection and primary anastomosis vs resection and stoma (ongoing).

New therapies are needed for surgical NEC and Dr Eaton described two novel strategies. Firstly, therapeutic hypothermia – cooling infants with NEC to as low as 33.5°C for a 48-hour period. A preliminary study confirms this is feasible and safe and a trial is now underway to see if there is any benefit to infants. The other exciting development is the use of stem cells in a neonatal rat model of NEC in which amniotic fluid stem cells were shown to improve survival and enhance repair of damaged intestine.

A network approach to reducing NEC: the East of England experience

Michele Upton described a quality improvement programme that was developed in response to concerns from clinicians in the East of England (EoE) about a perceived increase in the incidence of NEC in their local units. The NEC care bundle was designed and implemented across the 17 neonatal units in the EoE Perinatal Network with the ultimate aim of reducing the incidence of NEC. The care bundle comprised four elements:

1. Early promotion of expression to facilitate the use of maternal breast milk.
2. Ongoing support for expression and breastfeeding.
3. Following a standardised enteral feeding guideline (SFR).
4. Prevention of infection through the use of an aseptic non-touch technique (ANTT) for preparing milk feeds.

The project called for significant changes in practice across a wide geographical area and involved neonatal, midwifery and allied healthcare professionals[6]. Data collection is continuing to determine whether implementation has been successful in reducing the incidence of NEC but the programme has led to an increased use of expressed breast milk and increased breastfeeding rates at discharge in the EoE. There have also been improvements in data collection, compliance with the feeding guideline and milk preparation standards.

Development of intestinal immune regulation and NEC

Dr Jörn-Hendrik Weitkamp talked about the pathogenesis of NEC. He presented his work on T cell populations in the intestinal mucosa pointing out that there are significant differences between the prenatal development of T cells and T regulatory cells in humans and mice. In contrast to neonatal mice, even extremely premature human infants demonstrate an abundance of functional T regulatory cells in the mucosa of the small intestine resected for non-NEC surgical indications. In contrast the proportion of T regulatory cells is significantly reduced in the ileum of premature infants with NEC. This may contribute to the elevated inflammatory response that is characteristic of NEC and may predispose these infants to the condition[7].

Building on this, Dr Weitkamp presented evidence that increased expression of the gene Smad7 (an inhibitor of TGFβ signalling) in the blood of preterm infants with a history of chorioamnionitis, may predict an increased risk for NEC. He postulates that Smad7 overexpression leads to blockage of TGFβ signalling and a decrease in the proportion of T regulatory cells, which primes the premature intestine to an inflammatory state. Sustained intestinal barrier dysfunction may increase the risk for NEC upon secondary triggers such as viral infections, nutritional antigens or blood transfusion.

Blood transfusion and NEC: causation or association?

Before discussing the evidence for an association between red blood cell (RBC) transfusions and NEC, Professor David Paul reviewed the pathophysiology of anaemia of prematurity. Anaemia in preterm infants is common because of blood loss, a rapid turnover of RBCs, reduced ability to manufacture erythropoietin and greater need for an expanded blood volume for growth. Transfusions – frequent in low birthweight infants – can increase oxygen delivery, decrease apnoea and improve growth. However there is no adequate, clinically available measure of tissue oxygen demand and the decision to transfuse is left to clinical judgment.

Organiser Dr Minesh Khashu.

Professor Phillip Gordon.

One in 200 preterm infants who receive a blood transfusion will develop NEC, with up to 50% of cases occurring within 48 hours of transfusion. There is strong evidence of an association from retrospective studies and Professor Paul reviewed the main clinical questions that remain unanswered, for example the importance of:

- The haematocrit prior to transfusion
- The feeding regimen around the time of transfusion
- The age of the transfused blood

It is possible that the association between transfusion and NEC is confounded by other factors (illness severity, anaemia, recall bias) yet, despite no real evidence of causality between RBC transfusion and NEC, there are some potentially biologically plausible mechanisms:

- an antibody-mediated or inflammatory response akin to a TRALI (transfusion-related acute lung injury) reaction
- a cytokine response to RBC transfusion
- a nitric oxide-mediated mechanism for loss of physiological activity in banked blood.

Summary

The SIGNEC U.K. conference represents an important first-step in providing an international platform for a focused discussion on NEC. The event was well received by the multi-professional audience and is helping to foster collaboration between experts to facilitate improvements in practice and inspire research. To build on the success of this first meeting, SIGNEC U.K. intends to make this an annual event.

References

1. **Neu J., Mshvildadze M., Mai V.** A roadmap for understanding and preventing necrotizing enterocolitis. *Curr Gastroenterol Rep* 2008;10:450-57.
2. **Berman L., Moss R.L.** Necrotizing enterocolitis: an update. *Semin Fetal Neonatal Med* 2011;16:145-50.
3. **Rees C.M., Pierro A., Eaton S.** Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. *Arch Dis Child Fetal Neonatal Ed* 2007;92:F193-98.
4. **Pike K., Brocklehurst P., Jones D. et al.** Outcomes at 7 years for babies who developed neonatal necrotising enterocolitis: the ORACLE Children Study. *Arch Dis Child Fetal Neonatal Ed* 2012;97:F318-22.
5. **Been J.V., Lievense S., Zimmermann L.J., Kramer B.W., Wolfs T.G.** Chorioamnionitis as a risk factor for necrotizing enterocolitis: a systematic review and meta-analysis. *J Pediatr* 2013;162:236-42.
6. **Radbone L., Birch J., Upton M.** The development and implementation of a care bundle aimed at reducing the incidence of NEC. *Infant* 2013;9:14-19.
7. **Weitkamp J.H., Koyama T., Rock M.T. et al.** Necrotising enterocolitis is characterised by disrupted immune regulation and diminished mucosal regulatory (FOXP3)/effector (CD4, CD8) T cell ratios. *Gut* 2013;62:73-82.