SIGNEC UK Fourth International Conference on Necrotising Enterocolitis

The fourth SIGNEC conference, held in London in September 2016. Some delegates took the opportunity to explore Chelsea Football Club.

Introduction

SIGNEC UK was established as a special interest group for necrotising enterocolitis (NEC) by Professor Minesh Khashu to help advance a field of neonatal medicine that is poorly understood. NEC continues to be the major cause of mortality and morbidity for preterm infants. SIGNEC provides a platform for involvement of a wide range of healthcare professionals to facilitate knowledge sharing, networking and collaboration for optimising research and improvements in practice.

The fourth SIGNEC UK conference took place on 26-27 September 2016 at Chelsea Football Club, London, attracting a multidisciplinary audience of 157 delegates comprising neonatologists, paediatricians, neonatal surgeons, basic science researchers, neonatal nurses, parents, dietitians and representatives from charities and the healthcare industry.

Dietary supplementation with retinoic acid for prevention of NEC

Professor David Hackam from Johns Hopkins University opened the presentations by speaking on his group's research into 'bugs, germs and genes' in the pathogenesis of NEC. His laboratory focuses on the molecular causes of NEC to develop novel preventative and therapeutic approaches. His team has identified a critical role for the lipopolysaccharide signalling receptor, Toll-like receptor 4 (TLR4), in the pathogenesis of NEC. A premature infant has persistently elevated TLR4 signalling in the gut compared with a full term infant; a consequence of the expression of TLR4 on intestinal stem cells and its role in normal gut development.

In the postnatal period the persistently elevated TLR4 expression of the newborn becomes activated by dysbiotic microbiota, resulting in an exaggerated inflammatory response, increased intestinal epithelial injury and reduced repair. TLR4 signalling on the endothelium of the gut leads to impaired mesenteric perfusion. Interestingly, breast milk compounds, including epidermal growth factor (EGF), antagonise TLR4 signalling on the intestinal epithelium, providing insights into the protective properties of breast milk in this disease.

Recent studies have shown that TLR4 signalling in the intestinal epithelium leads to the recruitment of pro-inflammatory (Th17) lymphocytes and a reduction in anti-inflammatory (Treg) lymphocytes. However, the administration of retinoic acid (vitamin A), which induces Treg and reduces Th17 cells within the intestinal epithelium, has been shown to prevent NEC in mouse models.

Taken together, these findings provide clues to the development of NEC based on persistent expression of TLR4 and suggest that dietary modification, using agents such as retinoic acid, may have a beneficial role for this devastating disease. In his second talk on day 1, Professor Hackam focused on a mouse model of human NEC. On day 2 he gave a third presentation in which he eloquently described the evolution of surgical treatment for NEC.

The role of microRNAs in NEC

In the first of two presentations, Dr Misty Good summarised her research into the role of small, non-coding RNAs – microRNAs – in the pathogenesis of NEC. Her second presentation discussed the development of a mouse model of NEC utilising a combination of formula feeds, systemic hypoxia and bacteria isolated from the

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intestine of a preterm infant with NEC. Dr Good reviewed her previous investigations showing that breast milk is protective against NEC-induced intestinal inflammation by inhibiting TLR4 with the activation of EGF receptor (EGFR) expression in enterocytes. EGFR signalling protects against TLR4-mediated enterocyte apoptosis and enhanced enterocyte proliferation in a mouse model of NEC, thereby protecting the intestinal mucosal integrity and ameliorating the devastating effects of the disease. Given these findings, Dr Good's group sought to study the gene regulation of intestinal EGFR as it has potential to be a therapeutic or preventative target in NEC.

One mechanism of gene regulation involves microRNAs that bind to their corresponding messenger RNA to prevent mRNA translation with subsequent downregulation of genes. Dr Good's team found that microRNAs were differentially expressed in the intestinal tract of the premature infant with NEC; specifically, microRNA-17 was increased in the blood and intestine of premature infants with NEC. Infants with surgical NEC and increased

Day 1: Basic science and laboratory research	
Professor David J. Hackam *Chief of Pediatric Surgery, Johns Hopkins University, USA*	Dietary supplementation with retinoic acid for the prevention of NEC
Dr Misty Good *Assistant Professor of Pediatrics, University of Pittsburgh School of Medicine, USA*	The role of microRNAs in NEC
Dr Cheryl Battersby *Clinical Research Fellow, Imperial College London, UK*	The UK Neonatal Collaborative NEC Study
Professor Per Torp Sangild *Professor of Comparative Pediatrics and Nutrition, University of Copenhagen, Denmark*	The piglet model for spontaneous NEC mechanisms and clinically relevant interventions
Dr Misty Good *Assistant Professor of Pediatrics, University of Pittsburgh School of Medicine, USA*	The trifecta for a severe mouse model of NEC: formula feeds, hypoxia and NECteria
Dr Steven J. McElroy *Associate Professor, University of Iowa, USA*	The Paneth cell disruption model of NEC: a new pathway to study NEC
Professor David J. Hackam *Chief of Pediatric Surgery, Johns Hopkins University, USA*	A mouse model of human NEC with shared insights from non-human primates
Professor Gail E. Besner *Nationwide Children's Hospital, Columbus, USA*	Novel methods of stem cell therapy and probiotic administration for NEC
Panel discussion	Laboratory models of NEC
Day 2: Clinical research and improvements in practice	
Stephanie M. Vaughan *Co-founder and President, The Morgan Leary Vaughan Fund, USA*	The Morgan Leary Vaughan Fund: our family's story of survival, courage and strength
Professor Per Torp Sangild *Professor of Comparative Pediatrics and Nutrition, University of Copenhagen Denmark*	Optimal time, diet and progression of enteral feeds in preterm infants to avoid NEC and induce sufficient development
Dr Ruurd Van Elburg *Chief Scientific Officer, Early Life Nutrition, Nutricia Research, Netherlands*	New insights in human milk composition and benefits for preterm infants
Kate Branchett *Patient Voice and Insight Lead, NHS England West Midlands, UK*	Using the voice, experience and insight of families to drive improvement
Panel discussion	How can neonatal teams best support families whose infants are at high risk of, or develop, NEC?
Kate Tavener *Specialist Neonatal/Critical Care Dietitian, King's College Hospital, London, UK*	Nutritional management post NEC: a practical approach
Dr Narendra Aladangady *Consultant Neonatologist, Homerton University Hospital, London, UK*	Blood transfusion and intestinal perfusion in preterm infants
Professor Nigel Hall *Associate Professor of Paediatric Surgery, University of Southampton, UK*	The role of ultrasound in surgical decision making for NEC
Professor Gail E. Besner *Nationwide Children's Hospital, Columbus, USA*	An introduction to the NEC Surgery Trial (NEST, USA)
Professor David J. Hackam *Chief of Pediatric Surgery, Johns Hopkins University, USA*	Evolution of the surgical treatment of NEC: from drainage equipoise to real life
Professor Boris W. Kramer *Professor of Experimental Perinatology, Maastricht UMC, Netherlands*	The development of NEC and the gut-brain-axis: more than gut feelings!

TABLE 1 Programme of speakers.

microRNA-17 also displayed reduced expression of EGFR, decreased tight junctions and diminished proliferation markers in the intestine. Furthermore, Dr Good demonstrated that microRNA-17 specifically downregulated EGFR *in vitro* implying that microRNA regulation is a potential preventative or therapeutic target for NEC.

The UK Neonatal Collaborative NEC Study

Dr Cheryl Battersby (on behalf of the UK Neonatal Collaborative NEC Study Group) presented results of the UKNC-NEC study. The study quantified the incidence of the severest forms of NEC in England receiving surgery or leading to death in 2012-13; this figure was highest at 11% among those born at 24 weeks' gestation; there was no evidence for variation nationally across 23 neonatal networks. Propensity matching analysis showed that infants fed any own mother's milk in the first seven days *vs* none or later than seven days, had a reduced risk of NEC; infants fed any, compared to no bovine products in the first two weeks of life also had a lower risk of severe NEC, although effect sizes were small, and fortifier use in the first 14 days was low. A gestational-age specific case-definition for NEC surveillance and research developed using clinical and X-ray findings from over 3,000 infants was presented.

The piglet model for spontaneous NEC mechanisms and clinically-relevant interventions

Professor Per Sangild described his studies in preterm pigs documenting the detrimental effects of formula feeding, parenteral nutrition (PN) and excessive bacterial colonisation on NEC and sepsis risk. Conversely, there are benefits seen in early feeding with intact milk diets (eg mother's own milk) and certain formula supplements. The translational value of these results for infants should always be questioned but the preterm pig appears to be a useful tool in both basic and applied neonatology. In his second talk on day 2, Professor Per Sangild shared a large body of work on optimal time, diet and progression of enteral feeds undertaken over the last decade in his research facility.

The Paneth cell disruption model of NEC: a new pathway to study NEC

Dr Steven McElroy's laboratory at the University of Iowa has noted a lack of Paneth cells and Paneth cell products following development of NEC. Using this finding, his team has developed a novel mouse model of NEC that uses Paneth cell disruption followed by bacterial dysbiosis to induce injury that is consistent with the injury, inflammation patterns, and alterations in the bacterial microbiota that are seen with clinical disease. In his talk, Dr McElroy summarised this new model and discussed how it may be used to help answer some fundamental questions regarding the development of NEC.

Novel methods of stem cell therapy and probiotic administration for NEC

Professor Gail Besner reviewed several potential novel therapeutic strategies for the prevention of NEC. A growth factor known as heparin-binding EGF-like growth factor (HB-EGF) when administered enterally can protect the intestines from experimental NEC.

Further studies from the Besner Laboratory have investigated the administration of stem cells in the prevention of NEC. Recent findings demonstrate that mesenchymal stem cells and neural stem cells, derived from either amniotic fluid, bone marrow, or the intestine itself, all protect the intestines from NEC with equivalent efficacy. The team has now demonstrated that exosomes (nano-sized particles) secreted from these stem cells are as effective as the stem cells themselves in protecting the intestines from NEC; these exosomes may represent a non-cell-based therapy for NEC.

The panel discussion on laboratory models of NEC. From left: Professor Per Sangild, Dr Steven McElroy, Dr Misty Good, Professor David Hackam and Professor Gail Besner.

Besner and her colleagues have very recently identified a novel delivery strategy that can improve the ability of probiotics to prevent NEC. When the probiotic *Lactobacullus reuteri* is stimulated to produce a biofilm and then administered enterally, it can significantly reduce the incidence of NEC after just one single dose. All of the components of this delivery system are 'Generally Recognised as Safe' by the U.S. Food and Drug Administration, making this an exciting potential therapy for NEC.

The last session of the afternoon focused on the different laboratory models of NEC. This was an extraordinary opportunity for the panellists and audience as it was the first time that these researchers had shared a platform to discuss their various models.

The Morgan Leary Vaughan Fund: our family's story of survival, courage and strength

In the first presentation of day 2, Stephanie Vaughan, mother of a NEC survivor, shared how the unexpected birth of her twin sons at 28^{+1} weeks' gestation followed four days later by Morgan's diagnosis of NEC was the catalyst for founding The Morgan Leary Vaughan Fund. 'Morgan's Fund' is the first public charity dedicated to NEC in the USA; its mission is to promote public awareness and to advance research to prevent, diagnose, treat and cure NEC.

Ms Vaughan shared the charity's accomplishments and undertakings. It was the first patient organisation to advocate for NEC as a rare disease and the first to produce a disease-specific podcast series – Speaking of NEC – to educate clinicians and parents of very low birthweight infants, who have the greatest risk for developing NEC. Now, Morgan's Fund is developing a 'natural history study registry' for NEC in collaboration with the National Organization for Rare Disorders (NORD).

Human milk composition and benefits for preterm infants

Dr Ruurd Van Elburg shared his insights into various aspects of milk composition and highlighted areas that may benefit from further exploration.

Using the voice, experience and insight of families to drive improvement

Delegates heard from Kate Branchett about the importance of engaging with families, and the use of good communication tools and high quality information to help ensure shared decision making. This was followed by a panel discussion involving parents on how neonatal teams might better support families.

Nutritional management post-NEC: a practical approach

Specialist Neonatal/Critical Care Dietitian Kate Tavener, presented a practice review of feeding for infants post-NEC. Infants who develop NEC are often extremely preterm or growth restricted and therefore are already nutritional compromised. Goals of management include gut rest, preservation of nutritional status through the use of PN and the avoidance of liver damage. There is a significant lack of evidence in the area of feeding post-NEC and there is widespread variation in practice in terms of when to restart enteral feeds and which milk to choose in the absence of maternal breast milk. Feed choice ranges from the use of standard preterm feeds and hydrolysed preterm feeds to amino acid and peptide-based term formulas. Post-surgical management of NEC can be complicated by short gut and stomas; intestinal recovery can be protracted and require prolonged PN. Formula feed choice is made on an individualised basis, often using peptide or amino acid-based feeds. Ms Tavener illustrated these points using a case study.

Blood transfusion and intestinal perfusion in preterm infants

Professor Narendra Aladangady and co-researchers measured gut oxygenation using near infra-red spectroscopy, and blood flow using Doppler ultrasound in preterm infants receiving blood transfusion for clinical indication. They found that blood transfusion improved intestinal tissue oxygenation without altering intestinal blood flow velocity, irrespective of postnatal age, patent ductus arteriosus and enteral feeding.

Surgical decision making in NEC

Is there is a greater role for surgery in the care of infants with NEC? Professor Nigel Hall tackled the question of whether surgery earlier in the course of the disease would improve outcomes. A reliable indicator specifically identifying necrotic bowel could facilitate 'smarter' surgery; biomarkers and abdominal ultrasound could help clinicians to identify infants with NEC sooner.

One candidate biomarker, intestinal fatty acid binding protein (iFABP), is present at an increased level in infants with NEC compared to controls. Furthermore, infants with surgical NEC have higher levels than those managed medically. Other potential biomarkers include members of the claudin family and interleukin 8.

A number of groups have described typical sonographic findings in infants with NEC and data suggest that ultrasound can reliably identify infants with necrotic bowel with high sensitivity and specificity. Although ultrasound is non-invasive and relatively inexpensive, it is user-dependent and some units have limited availability and expertise.

An introduction to NEST, USA

In the second of two presentations, Professor Gail Besner reviewed previous randomised controlled trials in the USA and Europe that compared peritoneal drainage (PD) *vs* laparotomy for the surgical treatment of NEC, revealing no clear benefit from either treatment. However, neither trial assessed neurodevelopmental outcome in the survivors of NEC. An observation study suggested that laparotomy results in better neurodevelopmental outcome and this is the basis for the multicentre randomised NEC surgery trial (NEST), which was initiated in the USA in January 2010. The study will randomise over 300 patients requiring surgery for NEC to PD or laparotomy, and will include detailed assessment of neurodevelopmental outcome at 18-22 months after surgery. Randomisation is almost complete and neurological assessment is ongoing.

Conference organiser Professor Minesh Khashu.

Stephanie Vaughan.

Professor David Hackam.

The development of NEC and the gut-brain-axis

The gut-brain-axis represents a concept of interactions between the brain and the gut. The innervation of the gut comprises more than 100 million neurons and 90% of vagus nerve activity involves the gut. Certain microbiome changes in the gut are experimentally and clinically associated with adverse psychological development and impaired memory function.

Professor Boris Kramer's team analysed 19-year-old adolescents who were born at <32 weeks' gestation and/or with a birth weight of <1,500g. Characteristics at birth and neonatal disease history did not influence pain coping strategy in adolescence. Intelligence, however, moderated pain coping strategy in adolescents born preterm. The team analysed whether perinatal factors and pain coping strategy were associated with altered pain threshold, pain tolerance and pain intensity in adolescence; only NEC was associated with altered pain response in adolescents born preterm. This finding underlines the importance of adequate analgesia in newborn infants with NEC.

The gut microbiome has an effect on the contractibility and thus voiding of the gut. Clinical data have shown that the frequency of voiding is lower in preterm babies that later develop NEC. This suggests a role of the microbiome in the development of NEC that might be anticipated by stool frequencies.

Summary

As in previous years, the fourth SIGNEC UK conference was well received by the whole audience who fed back high praise for the quality of presentations and discussions. The degree of involvement from parents and families is quite unique and serves the clinicians and researchers well.

For updates on the fifth SIGNEC conference, planned for 16-17 October 2017 at Chelsea Football Club, London, contact mineshkhashu@gmail.com