

Etiopathogenesis of necrotizing enterocolitis

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Chapter 10

VALORISATION ADDENDUM



RELEVANCE

Necrotizing enterocolitis (NEC) is an inflammatory intestinal disorder primarily seen in preterm infants, characterized by variable damage to the intestinal tract, ranging from mucosal injury to full-thickness necrosis and perforation.¹⁻⁵ NEC remains a leading cause of morbidity and mortality in neonatal intensive care units. The overall incidence of NEC is about 1 in 1000 live births, but occurs in up to 7-11% of very low birth weight (VLBW, birth weight < 1500 g) infants. Mortality is 15-30% and is higher with lower birth weight and earlier gestational age.

The clinical picture of NEC includes distended abdomen, peri-umbilical erythema, bloody stools, feeding intolerance and overall instability of the infant. The modified Bell's staging criteria^{6,7} are used to stratify NEC in 3 different stages of increasing severity. This staging system uses systemic signs, findings in the abdominal examination, and radiographic clues. In NEC Bell's stage I there are non-specific symptoms as in feed intolerance and non-gut related sepsis. However, these infants can rapidly progress to stage III. Bell's stage II and stage III indicate definite and advanced stages of the disease respectively. Intestinal perforation is defined as stage IIIB.⁸

Early detection and initiation of treatment in NEC are key factors in its course and prognosis. Basic principles of medical management of NEC include bowel rest, antibiotics, routine gastric decompression and supportive care.⁸ Pneumoperitoneum due to intestinal perforation is an absolute indication for surgery. However, there are also some relative indication for surgery in NEC.⁹

Survivors may be left with significant sequelae, which include gastrointestinal complications such as a short gut syndrome, but also stoma related morbidity, strictures, and central venous catheter related problems (for example sepsis).^{5,9}

NEC is a disease associated with a prolongation of the NICU stay. Both medical and surgical NEC markedly increase the costs of hospitalization.^{5,10} It is estimated that the average charge in the United States associated with a case of surgical NEC is

\$400.000 to \$500.000. One prospective study, which considered hospital cost, found that the costs of surgical NEC were between \$300 000 and \$660 000.¹¹

The long-term outcome after hospital discharge was evaluated in several studies. NEC is associated with significantly worse neurodevelopmental outcome (both motor and cognitive outcome) early in life¹² and at school age than prematurity alone.¹³⁻¹⁵ Presence of advanced NEC and need for surgery increase the risk of neurological impairment.¹⁴ This is not only at early school age, but also educational outcome at 11 years is worse.¹⁶ Also NEC survivors demonstrated significant long-term consequences of gut function (presence of stoma, admission for bowel problems and continuing medical care for gut-related problems).¹⁵

Obviously preventive strategies that reduce the prevalence of NEC during the NICU hospitalization are both a clinical and an economic priority for society. This can only be achieved with a more profound understanding of the pathophysiological mechanisms leading to NEC. One of the suspected etiopathogenic mechanisms is an immature mesenteric circulatory regulation. Understanding of the mechanisms that regulate the mesenteric artery (MA) vascular tone, could have clinical implications and lead to better management options and better neonatal outcome.

Significant progress in our understanding of the cardiovascular physiology and pathophysiology has been achieved with the use of animal models. However, mammalian models are complex because the fetal/placental circulation has to be exposed to intervention only through complex surgery and experimental manipulations affect both the mother and the fetus. Therefore, there is a need for additional models, addressing these limitations.¹⁷ The chicken (*Gallus gallus*) embryo represent an excellent model for investigating developmental physiology of the cardiovascular system.¹⁸ Chicken embryos have a mammalian-like circulation, with an extraembryonic circuit involved in the gas exchange (the chorioallantois), analogous to the placenta. In contrast to mammals, which depend on a continuous transference of nutrients from the maternal circulation to the developing fetus, all the nutrients required for the formation and growth of the chicken embryo are pre-packaged in the egg at the time of laying.¹⁹ As the chicken embryo develops outside the mother, the number of experimental animals is divided by two, and the effects of external stresses on

cardiovascular development can be studied without interferences of maternal hormonal, metabolic, or hemodynamic alterations.

INNOVATION

With this present thesis, we focused on role of the vascular system in the pathogenesis of NEC and approached the problem from two perspectives. In the first part, we investigated the developmental changes in mesenteric arterial reactivity. In the second part, we analyzed the role of L-arginine, the precursor for the synthesis of NO, on the pathogenesis of NEC.

Only a limited number of observations have been made on the development of fetal intestinal circulation in mammals. In the chicken embryo only relative immature vessels were investigated without analyzing further maturation. Our publication described the maturational differences in the reactivity of MAs isolated from chicken embryos at several stages and hatchlings. To this knowledge we added the evidence that chronic moderate hypoxia during incubation results in subtle but significant alterations in chicken MA reactivity, small intestine morphology and VEGF expression. To the best of our knowledge, no study has investigated the response to hypoxia in isolated fetal MAs and even the data in neonatal MAs are scarce. We are the first showing that the local response to hypoxia was absent in the less mature MA, indirectly support the hypothesis that the immature intestine is less susceptible to direct hypoxic damage. In fact, the publication of our findings has already attracted the attention of other investigators in the field of MA developmental biology.^{20,21}

The use of animal subjects is fundamental to the advancement of biomedical research and practice, at least until viable replacements are found. Equally important are the ethical guidelines and welfare protocols that shape the conduct of such research. Animal welfare guidelines and legislation still emphasises Russell and Burch's 'three R's': reduction (of the number of animals used), refinement (of testing procedures to minimise suffering), and replacement (of animal models with alternatives).²²

L-arginine is the substrate for NO production and several studies demonstrated that plasma arginine concentrations are decreased in infants with NEC. Nevertheless, the mechanisms explaining this relative deficiency of L-arginine in infants with NEC are far from being understood. Several studies demonstrated the association of the CPS1 p.Thr1406Asn genotype with clinical situations where endogenous NO production is critically important. But the role of this polymorphism in NEC was not investigated until our retrospective case-control study and recent multicenter, prospective cohort study. We provided evidence that a functional variant of the CPS1 gene may contribute to NEC susceptibility. Even in the recent literature about NEC, our retrospective case-control study from several years ago is cited. Last but not least, our data did not confirm the hypothesis that in a population of preterm infants, in whom the urea cycle is not fully developed, genetically determined variations in CPS1 function would induce further changes in L-arginine levels. We did not find differences in urea cycle intermediates (i.e. citrulline and arginine) between the different CPS1 genotypes.

In conclusion, this thesis adds understanding of the basic mechanisms of normal and altered functional and structural development of the mesenteric vessels to the current knowledge about MA vasoreactivity. This, as well as inter-species differences in mesenteric circulation, may provide insights into human intestinal disease.

Also this thesis provides further evidence that a functional variant of the CPS1 gene may contribute to NEC susceptibility. This brings us one step closer to developing a laboratory genetic test that could predict, when environmental factors are properly assessed, the risk/probability of preterm infants developing NEC and leads to more targeted therapies and also preventive strategies. This is important to reach the ultimate goal reducing the prevalence of NEC during the NICU hospitalization.

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