Introduction

Necrotizing enterocolitis (NEC) is a disease of the gastrointestinal tract of neonates which finally results in inflammation and bacterial invasion of the bowel wall **(Lin & Stoll, 2006).**

Although there is extensive research, the pathophysiology of NEC is still unclear and therapeutic options are limited **(Yajamanyam et al., 2014)**.

Clinical manifestations of NEC may be vague, including increased episodes of apnea, desaturations, bradycardia, lethargy and temperature instability. There may be GI-specific symptoms as feeding intolerance, bloody stools, emesis, abdominal distention, tenderness and abdominal wall discoloration **(Thompson & Bizzarro, 2008).**

Radiographic signs may include ileus, dilated or fixed intestinal loops, air in the intestinal wall or free air in the abdomen. NEC diagnosis, however, remains challenging because many now see that Bell's staging criteria **(Bell et al., 1978)** used for diagnosis as being not accurate **(Gordon et al., 2007).**

Neutrophil to lymphocyte ratio (NLR) is used as a marker of subclinical inflammation. It is calculated through dividing the number of neutrophils by number of lymphocytes, usually from peripheral blood sample **(Yang et al., 2018)**.

Increased neutrophil proportion reflects the deterioration of the immune system only, whereas decreased lymphocyte count reflects the increasing level of physical stress. Moreover, NLR is closely related to the inhibition of body's immune function **(De Jager et al., 2010).**

In a word, NLR could indicate the status of body's inflammation response and the level of physical stress timely and accurately **(Yang et al., 2015).**

In a clinical study conducted in 2001, the authors suggested the routine use of NLR as a stress factor in clinical ICU practice, and they claimed that NLR might has a prognostic and a predictive value of many diseases **(Zahorec, 2001)**.

Gamma glutamyl transferase (GGT) is an enzyme found in the cell membranes of many tissues. The highest concentration is in the kidney, but the liver is considered the source of normal enzyme activity **(Fischbach & Dunning, 2003)**.

GGT transfers the amino acids across the cell membrane **(Bell et al., 1978)** and in leukotriene metabolism **(Cabrera-Abreu & Green, 2002)**.

It has an intracellular antioxidant role as it is involved in glutathione metabolism, resulting in the cysteine formation **(Yokoyama, 2007)**. GGT is removed from the plasma by liver uptake.

Bilirubin is a free radical remover with anti-inflammatory and antioxidant role (Stocker et al., 1987).

One report described that stage III NEC had lower total serum bilirubin than their mild or disease-free controls during the first 14 days of life **(Fereshtehnejad et al., 2012).**

As serum bilirubin level is a function of the activity of UGT1A1, albumin binding, and the bilirubin load **(Levitt, 2014);** it will be necessary to find if serum bilirubin level links to NEC development or severity.

Relatively constant serum calcium (Ca2+) level is essential for cellular function and under firm control by the neural/humoral factors. It is not clear what makes serum Ca2+ lower in NEC neonates. In a recent report, higher level of serum GGT, a lower serum bilirubin and Ca2+ were found in severe NEC **(Han & Wei,** ***2017)***.

Aim of the Work

To assess the value of peripheral blood neutrophil to lymphocyte ratio (NLR), serum levels of γ-Glutamyl transferase (GGT), total serum bilirubin and serum calcium (Ca2+) concentrations for early diagnosis as well as prediction of NEC severity and if found significant, scoring will be done according to their levels in different modified Bell's stages.

Chapter 1

Neonatal Necrotising Enterocolitis

Definition:

Necrotizing enterocolitis (NEC) is an inflammatory intestinal disease that affects mostly preterm neonates **(Neu & Walker, 2011)**.

It can cause variable intestinal injury from epithelial injury to transmural involvement and perforation and is marked by inflammation and bacterial invasion **(Thompson & Bizzarro, 2008).**

NEC is an important source of morbidity and mortality in preterm infants **(Cotten et al., 2005)**.

It affects approximately 10% of preterm patients who has a birth weight of less than 1,500 grams **(Luig & Lui, 2005)**.

The mortality rate for preterm infants who have extremely low birth weight (less than 1,000 grams) is 30–50% and for infant with a very low birth weight (VLBW) (<1,500 grams) is 10–30%, and there has been an insignificant change in the past 20 years **(Fitzgibbons et al., 2009)**.

To evaluate the grade of NEC, Bell's classification was suggested in 1978 **(Bell et al., 1978)**. It sorts the grade of NEC based on clinical and radiographic signs and rests the most commonly used method in early assessment.

In latest years, however, this staging criteria has been changed as our information about the disease has improved, however there is a debate concerning the validity of this staging classification at lower gestational ages (GAs) **(Gordon et al., 2007).**

Severity of NEC is considered a key role in both the management and the outcome of affected neonates, so neonates who have proven or advanced NEC, classified as Bell's stage II and III, respectively, are at high risk of developing peritonitis, sepsis, bowel perforation, and additional severe systematic problems including multi-system organ failure and capillary leak syndrome **(Sonntag et al., 1998).**

The pathophysiology of NEC is multifactorial and still not totally understood. There are numerous risk factors for the development of the disease, but some are controversial, so there is a difficulty in creating new approaches to stop the development of NEC and the progression of it.

Epidemiology:

The incidence of established NEC (Bell's stage II and III) in preterm babies is influenced by the degree of prematurity and the geographic place of the patient. A systematic review was done on the incidence of NEC in high-income states found difference in the incidence of NEC based on birth weight, GA, and country **(Battersby et al., 2018)**.

Overall, the incidence of NEC was maximum among the most preterm infants.

In infants born at a GA of less than 28 weeks, the lowest informed incidence of NEC was in Japan (2%) and the highest was in Canada, Australia and Italy (7–9%) **(Battersby et al., 2018)**.

In neonates with a GA of between 28 and 31 weeks, informed NEC incidence was also lowest in Japan (0.2%), while other developed states had incidence rates ranging from 2–3% **(Battersby et al., 2018)**.

Similarly, for VLBW infants, NEC incidence ranged from 2% in Japan to 6–7% in the USA and 9% in Poland **(Battersby et al., 2018).**

These outcomes collectively show that the degree of prematurity and low birth weight are essential factors in developing NEC. The various incidence rates between states suggest numerous factors affecting the development of NEC including environment, genetic predisposition and diet **(Battersby et al., 2018)**.

Risk factors:

The only consistently defined risk factors for NEC are formula feeding, prematurity, intestinal dysbiosis and low birth weight **(Rose & Patel, 2018)**.

Prematurity and low birth weight are the most frequently reported risk factors for NEC, with the lowest birth weights and GAs having the highest incidence of NEC **(Samuels et al., 2017)**.

Maternal causes such as chorioamnionitis, lack of prenatal steroids, cocaine abuse, mode of delivery, in-utero growth restriction, placental abruption, high body mass index, pre-eclampsia, intrahepatic cholestasis through pregnancy, and smoking have been involved in the development of NEC (**Travers et al., 2017; Lu et al., 2017)**.

Moreover, a lot of further risk factors for the NEC development have been reported such as administration of acid-suppressing medications, antibiotic exposure, acute hypoxia, cardiac anomalies, blood transfusions, poor intestinal perfusion, and neonatal anemia **(Denning & Prince, 2018)**.

Yet, the incidence of NEC was not lesser in infants who went through primary surgical closure of PDA compared to infants who were given indomethacin **(Yee and Scotland, 2012).**

Pathophysiology:

NEC affects many organs and its pathophysiology is multifactorial. The classical understanding of NEC pathophysiology was that intraluminal bacteria disrupt and attack the intestinal epithelium at the tips of intestinal villi **(Zhang et al., 2012)**.

Endotoxin from these bacteria binds to Toll-like receptor 4 (TLR4) found on the intestinal epithelial cells, stimulating pathogen-associated molecular pattern (PAMP) receptors, which help the break-down of the gut barrier as well as permit bacteria to translocate **(Molteni et al., 2016).**

This process subsequently cause an intense inflammatory response in the lamina propria mediated by tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and other inflammatory cytokines **(De Plaen, 2013)**.

Vasoactive substances are also released in the intestine, and those related to NEC such asendothelin-1 (ET-1), platelet-activating factor (PAF), and nitric oxide (NO) **(Schnabl et al., 2008).**

Complement as well as coagulation systems are also stimulated by the intestinal inflammation. Leukocytes and platelets in these systems adhere to the endothelium, inhibiting blood flow in the micro vasculature of the small intestine and result in tissue injury. Extra damage to the endothelium from adherent neutrophils and platelets also impairs NO generation required for vaso relaxation **(Claud et al., 2013; Watkins & Besner, 2013).**

Through the efforts of numerous research laboratories, potential methods have been explored to investigate how the disease may develop and how it can be treated.

Nitric oxide:

The pathogenesis of NEC is possibly started by postnatal insults on the immature intestine in the existence of a number of the previously declared risk factors. These factors cause the initial epithelial injury, which triggers an intestinal release of inflammatory mediators and inflammatory response **(Nowicki et al., 2006).**

Ford et al. **(Ford et al., 1997)** first discovered the role of NO and inducible NO synthase (iNOS)-derived NO in NEC occurrence.

NO has an important role in NEC development that have been widely explored. Once intestinal barrier fails, the lamina propria is exposed to great levels of endotoxins and other bacterial products that cause bacterial translocation **(Ciftci et al., 2012).**

The final result is stimulation of the neonatal immune system and start an inflammatory cascade which causes a strong pro-inflammatory response by the release of prostanoids, NO, and cytokines. NO interacts with superoxide and produces peroxynitrite, a strong oxidant **(Aceti et al., 2018)**.

This afterwards leads to enterocyte apoptosis or necrosis and impairment of both enterocyte proliferation and epithelial repair done by enterocyte migration. The imbalance between tissue injury and repair further stimulates the inflammatory cascade and NEC development. The net result of these insults is more epithelial damage and bacterial translocation that causing sepsis, a vicious cycle which leads to sever inflammation, bowel necrosis, perforation, and death **(Chokshi et al., 2008).**

Toll-like receptor 4:

The intestinal epithelium has a high expression of TLR4 in premature infant **(Hackam & Sodhi, 2018)**.

TLRs are important in the triggering of innate immunity by identifying specific forms of microbial components **(Takeda & Akira, 2004).**

TLR4 expression was high in mice and humans with NEC **(Egan et al., 2016)** and they have found mutations in the TLR4 signaling pathways in human NEC (**Fawley et al., 2017).**

Moreover, TLR4 knockout mice were safe from NEC development **(Sodhi et al., 2012)**.

TLR4 is stimulated by lipopolysaccharides part on Gram-negative bacteria **(Hackam & Sodhi, 2018)**.

Intestinal lumen microbes Activate TLR4 and result in impaired intestinal repair and barrier injury **(Leaphart et al., 2007)**, which consequently permits the translocation of the luminal bacteria, vasoconstriction which leads to intestinal ischemia, and NEC **(Lu et al., 2014).**

TLR4 can also be suppressed by probiotic bacteria that stimulate TLR9 **(deKivit et al., 2014)** and can stop goblet cell differentiation **(Sodhi et al., 2012)**, which are important to preserve the physical mucous intestinal barrier to pathogenic bacteria.

Microvascular blood flow:

NEC is often characterized by areas of intestinal ischemia with inadequate blood supply. Generalized neonatal hypoxia and exchange transfusions are strongly linked with the development of NEC **(Beeby and Jeffery, 1992)**.

Imbalance of the intestinal micro-circulation in NEC leads to zones of poor blood flow which may help the activation of the inflammatory cascade and cause intestinal injury **(Young et al., 2011)**.

Postprandial hypoxia and feeding have been proved to synergistically prompt intestinal hypoxia in experimental NEC, highlighting the essential balance between oxygen supply and demand **(Chen et al., 2016)**. The importance of circulation in the pathogenesis of NEC is still an area of research interest.

Microbiota:

Dysbiosis is a disruption of gut microbiota growth and its homeostasis, which has been related to the development of NEC **(Cho & Blaser, 2012).**

This pathological process includes a deficiency of useful commensal microbiota in addition with a low diversity of bacteria, which permits the overgrowth of pathogenic bacteria that activate the inflammatory cascade **(Patel & Denning, 2015).**

A meta-analysis of intestinal dysbiosis in preterm infants preceding NEC found a high relative abundance of Proteobacteria as well as a low relative abundance of Firmicutes and Bacteroides **(Pammi et al., 2017).**

Dysbiosis has been related to the usage of antacids and/or antibiotics in the NICU, inflammatory response dysregulation and formula feeding **(Cassir et al., 2016).**

NEC and matched control fecal samples tested for bacterial diversity and clustering revealed that NEC patients are likely to have less diverse microbiomes and different distributions of the intestinal bacteria **(Morrow et al., 2013; Torrazza et al., 2013).**

Proteobacteria may initiate sever inflammatory response and colonization by anaerobic bacteria, that have been related to NEC **(Torrazza & Neu, 2013).**

However, as Proteobacteria are also common constituents in the intestinal microbiome of preterm neonates who do not have NEC, the role of microbiota is still vague and is still only part of the complex pathogenesis **(La Rosa et al., 2014).**

Prophylactic probiotics have been proved to decrease the NEC development when baseline incidence levels are high **(Hackam et al., 2013)**, not all probiotics are equally effective **(Patel & Underwood, 2018).**

Comparing the efficacy of various strains, combinations of various strains and long-term safety is still areas of present research **(Chang et al., 2017; Dermyshi et al., 2017).**

Intestinal stem cells and epithelial regeneration:

The small intestinal epithelium regenerates every three to six days, a rate driven by dynamic proliferation inside the intestinal crypts towards the villus tip **(Gassler, 2017).**

Within the crypts there is a special stem cell zone which is having intestinal stem cells (ISCs). ISCs are vital for creating progenitor cells that differentiate into various types of epithelial cells like enterocytes, entero-endocrine cells, Paneth cells, and goblet cells **(Umar, 2010).**

ISCs are activated and replicate in response to intestinal injury. To calculate the number of ISCs, leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5), a well-established wingless integrated (Wnt)-associated stem cell marker **(Khan et al., 2017)** has been used.

There is a decrease in Lgr5-positive ISCs in NEC patients **(Gassler, 2017; Khan et al., 2017; Niño et al., 2017)** and restoration of ISC activity seems like to have a useful effect, yet the way by which ISC viability is disturbed in NEC is still poorly understood and is now being investigated **(Niño et al., 2017)**.

Diagnosis:

Suspected or confirmed NEC infants are those who met even one of historical, clinical or radiological criteria for NEC diagnosis according to Walsh and Kliegman modified Bell's Staging criteria **(Walsh & Kliegman, 1986)** as shown in **Table 1**.