Transfusion Practices in Very Low Birth Weight Neonates and the development of Necrotising Enterocolitis in two Neonatal Units in Bloemfontein, Free State.

Author:

Dr I Kruger

Student Number: 2006013698

Supervisor:

Dr E Brits

Consultant Paediatric Surgeon

Department of Paediatric Surgery

Faculty of Healthy Science

School of Medicine

University of the Free State

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Declaration

I, Iselma Kruger, hereby declare that the minor dissertation titled “Transfusion Practices in Very Low Birth Weight Neonates and the Development of Necrotising Enterocolitis in two Neonatal Units in Bloemfontein, Free State”, is my own work and has not been submitted previously by me to this, or any other tertiary institution. I furthermore declare that all sources which I have used or quoted, have been indicated and acknowledged with complete references.

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Abstract:

Background:

Necrotising enterocolitis (NEC) places a massive burden on neonatal units and the healthcare system. Not only does it significantly prolong the hospital stay of neonates, it also causes detrimental long-term sequelae, such as neurodevelopmental delay, growth retardation and chronic gastrointestinal complications. However, despite it being a well-documented disease, with the first reports of clinical findings indicative of NEC being made as early as the early 1800's, it continues to elude neonatologists in terms of risk factors, prevention and treatment strategies.

Further research is necessary to help identify possible contributing factors, to improve treatment, as well as to develop preventative strategies for this difficult condition. One possible contributing factor that has been identified by researchers, is the transfusion of blood products in the neonatal period, especially in preterm neonates. Since there are currently no standardised Red Blood Cell (RBC) transfusion protocols in either of the two Neonatal Intensive Care Units (NICU's) in Pelonomi Tertiary Hospital (PTH), and Universitas Academic Hospital (UAH), patients receive RBC transfusions at a great variety of different clinical stages.

Objectives:

By retrospectively evaluating the RBC transfusion practices in these units, specifically in those patients who developed NEC, we hoped to gain better insight into the possible causative relationship between RBC transfusions, and the development of NEC in Very Low Birth Weight (VLBW) neonates.

Method:

Data was collected on a total of 1585 VLBW neonates who were treated at PTH, and UAH, during a retrospective 5-year period. Data collected included gestational age, birth weight, RBC transfusion data, and data regarding the development of NEC. The RBC transfusion data included their pre-transfusion haematocrit, post-transfusion haematocrit, recorded clinical state during transfusion, ventilatory state during transfusion, and whether they were kept nil per os (NPO) during, and for at least 120minutes after the transfusion.

Regarding NEC: for all VLBW neonates who developed NEC the following data was recorded: The Modified Bell's Staging grade, whether they developed NEC before an RBC transfusion, or within 48-hours after an RBC transfusion.

Results:

This study showed that the incidence of NEC in VLBW neonates in these two academic hospitals was higher than the expected international number. There was a definite decrease in the number of VLBW neonates who received RBC transfusions over the 5-year period, with an improvement in feeding practices during transfusions. This decrease in RBC transfusions correlated with a decrease in the incidence of Transfusion Associate Necrotising Enterocolitis

(TANEC), but no statistical significance between feeding practices during RBC transfusion and the development of TANEC, could be demonstrated.

Definitions

Definition of Terms:

Neonate:

Any new-born baby up to the age of 28 days.[1]

Preterm Neonate:

Any neonate born to a mother who has been pregnant for less than 37 weeks.[1]

Necrotising Enterocolitis (NEC):

Necrotising enterocolitis is necro-inflammatory cellular death (necrosis) of the bowel of a neonate, diagnosed by utilizing clinical and radiographic findings, as described in a later section (Diagnosis).

Transfusion Associated Necrotising Enterocolitis (TANEC):

NEC that occurs in a patient within 48-hours after receiving a transfusion of red blood cells.[2]

Very Low Birth Weight Neonates (VLBW):

Any neonate born at a weight of between 1000g and 1499g.[2]

Enteral Feeds:

Nutritional substances (feeds) delivered to the patient directly via the gastro-intestinal tract (thus either via the mouth, or directly into the stomach or small intestine).[2]

Red Blood Cells (RBC's):

Donor red blood cells that have been separated to be used as a blood transfusion in patients.[3]

List of Abbreviations

NEC:	Necrotising Enterocolitis
NPO:	nil per os
PAF:	Platelet activating factor
PTH:	Pelonomi Tertiary Hospital
RBC's:	Red Blood Cell's (as pertaining to a transfusion)
TANEC:	Transfusion Associated Necrotising Enterocolitis
TNF:	Tumour necrosis factor
UAH:	Universitas Academic Hospital
VLBW:	Very Low Birth Weight (as pertaining to a neonate)

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Chapter 1: Introduction

1.1 Motivation for this study

Necrotising enterocolitis (NEC) places a massive burden on neonatal units and the healthcare system. Not only does it significantly prolong the hospital stay of neonates, it also causes detrimental long-term sequelae, such as neurodevelopmental delay, growth retardation and chronic gastrointestinal complications[1]. However, despite it being a well-documented disease, with the first reports of clinical findings indicative of NEC being made as early as the early 1800's [4], the exact aetiology it continues to elude neonatologists, although risk factors, and prevention and treatment strategies have been investigated and identified.

Further research is necessary to help identify possible contributing factors, to improve treatment, as well as to develop preventative strategies for this difficult condition. One possible contributing factor that has been identified by researchers, is the transfusion of blood products in the neonatal period, especially in preterm neonates. Enteral feeding during the transfusion of blood products has also been identified as a significant modifiable risk factor[5]. Since there are currently no standardised RBC transfusion protocols in the two Neonatal Intensive Care Units (NICU's) in PTH, and UAH, patients receive RBC transfusions at a great variety of different clinical stages.

By retrospectively evaluating the RBC transfusion practices in these units, specifically in those patients who had developed NEC, we hoped to gain better insight into the possible causative relationship between RBC transfusions and the development of NEC.

1.2 Research Purpose

1.2.1 Primary Objectives

The aim of this study is to evaluate the RBC transfusion practices in VLBW neonates in two academic neonatal units in Bloemfontein, Free State, in correlation with International Guidelines by:

- Comparing and describing the RBC transfusion practices between the two hospitals
- Recording any changes in RBC transfusion practices, over a 5-year period
- Evaluating possible differences in outcomes, specifically pertaining to NEC

1.2.2 Secondary Objectives

Additionally, we aim to gain further insight into the impact of RBC transfusion practices in VLBW neonates by evaluating:

- The haematological indications for RBC transfusions
- The clinical state of neonates during RBC transfusions

- Whether or not they were receiving enteral feeds during the RBC transfusion
- The possible association between the RBC transfusions, the timing of enteral feed in relation to the RBC transfusion, and the development of NEC as well as the severity thereof

By evaluating our practices over a 5-year period and being able to identify possible shortcomings in our RBC transfusion protocols, we hope to significantly improve our clinical practice.

1.3 Structure of this dissertation

Chapter 1 serves to introduce readers to the study in context of the current literature whilst outlining the aims, objectives, and potential contributions to the field.

Chapter 2 presents a concise, yet thorough review of the important literature published regarding this topic as well as internationally accepted practices. A definition of the most important terms, epidemiological review, description of the pathophysiology, methods for making the diagnosis, preventative strategies and management strategies will be discussed. A brief description of Transfusion Associated Necrotising Enterocolitis (TANEC) and the theories regarding this is also discussed as background and motivation for this study.

Chapter 3 will describe the research question and outline the problem statement and aim of this study.

Chapter 4 outlines the specifics regarding the research design of the study, methods used for data collection, statistical analysis, and ethical considerations and approval received.

Chapter 5 presents all the results obtained from the data collected, outlining specifically the general epidemiological data, the RBC transfusion results, and NEC results for both PTH and UAH separately, and a comparison between the two institutions. A review of the RBC transfusion practices as evaluated over 5 years, as well as the incidence of NEC over the 5 years is also compared.

Chapter 6 is a brief discussion of the results in the context of the latest literature, comparing the results of our study to results from other studies around the world.

Chapter 7 will lastly aim to bring the results as outlined in chapter 4, into context with the current literature, present the final conclusions of the study and delineate potential areas of future research.

Chapter 2: Literature Review

2.1 Introduction

Although NEC as a disease entity has been well known and documented since the early 1950's, it is still a clinical conundrum.[6] Since it has proven to be one of the most common and deadliest surgical emergencies in the neonatal population, large volume research has been conducted in the past couple of decades.[7]

This chapter will present the current available literature on NEC, and the potential link between NEC and RBC transfusions. The aim of this chapter is to provide some in-depth background information regarding these topics, and to provide the groundwork of knowledge for understanding this study.

2.2 Epidemiology

The incidence of NEC is estimated to range from 0.4 and 1.8 in 1000 live births.[8,9] This incidence increases with a decrease in birth weight, being as high as 11% in VLBW neonates.[10] However, the incidence of NEC seems to vary greatly between different institution and countries, specifically traditionally thought to be of importance was the difference between low to mid income countries, and high-income countries. The National Institute of Child Health and Human Development recorded a variation of between 4% and 20% at institutions across the USA.[11]

A more recent systematic review conducted by Alsaied et al, estimated the incidence of NEC in VLBW neonates to be 7%. They reviewed 27 cohort studies that reported the incidence of NEC, from both low to mid income countries, and high-income counties. There was no statistically significant difference recorded in the incidence of NEC between the different income countries, but a marked increase in the incidence of NEC was noted over the years. They also suggested that more studies in low to mid income countries are needed.[12]

Since the term "necrotising enterocolitis" was first used in 1953 by Schmid and Quaiser,[6] there has been a vast increase in the number of neonates diagnosed with this condition. This increase in incidence is attributed both to the increased number of premature babies delivered via Caesarean section, as well as the improvement in neonatal intensive care, ensuring the survival of more premature and lower birth weight neonates, who are at a higher risk for developing NEC.[11]

NEC associated mortality ranges between 15 and 30%, with birth weights and mortality rates being inversely proportionate.[13,14,15] There is no proven definitive prevention or cure for NEC as yet, and approximately 35 - 50% of patients with NEC will require surgical intervention, while the rest can be managed medically.[16,14,17] Mortality, as well as long term sequalae such as neurodevelopmental delay, growth retardation and chronic gastrointestinal complications are increased in patients who require surgical intervention.[18]

Most neonates who develop NEC are born preterm, but it sometimes also occurs in term neonates, with up to 12% of all NEC cases occurring in the latter group.[11] For the purpose of this

study we will be focussing specifically on preterm neonates who developed NEC, as the pathophysiology and aetiology of NEC in term neonates are thought to be different.

In addition, NEC poses a large social-economic burden leading to considerably longer hospitalisation times. In one study this translates into as much as 60 days longer length of stay for neonates with NEC who required surgical intervention.[19] Especially in lower income countries this is a heavy economic burden to bare.

2.3 Pathophysiology

Active research is ongoing to gain a more complete understanding of the aetiology and pathophysiology of NEC. At tissue and cellular level, NEC seems to be an inflammatory process, with overgrowth of bacteria, and necrotic changes. Worsening of the aforementioned pathological processes, can lead to air in the subserosal and submucosal layers of the intestinal wall (pneumatosis intestinalis), air in the portal venous and lymphatic systems, and complete disruption of the intestinal wall leading to intestinal perforation and pneumoperitoneum.[2] The pneumatosis is due to nitrogen and hydrogen produced by gas producing bacteria in the gastro-intestinal tract.[20] Histologically the bowel of neonates with developing, or early NEC will show microthrombus formation with resultant transmural oedema, patchy ulceration, and haemorrhage.[21]

The single common denominator seems to be the presence of an excessive inflammatory response (probably due to an immature intestinal immunity) to a yet ill-defined insult. Some of the postulated possible causes for this response includes overgrowth of normal bacteria, ischemia, infection due to abnormally localized bacteria, and different feeding strategies.[22]

2.3.1 Ischemic changes

Intestinal circulation is controlled by both extrinsic and intrinsic factors. The autonomic nervous system and cardiovascular reflexes extrinsically control splanchnic blood flow, by diverting blood flow away from the intestinal circulation in an attempt to preserve systemic perfusion. In addition, the intestines form different mediators that have an intrinsic control over the local blood flow.[23]

Resting vascular resistance is the biggest factor that influences intestinal microcirculation. As per the Hagen-Poiseuille equation the rate of flow in a vessel is proportional to the radius of the vessel to the fourth power. This means that small changes in vascular diameter, due to vasodilatation or vasoconstriction, leads to much larger changes in vascular resistance and blood flow.[23] As mentioned the autonomic nervous system and cardiovascular reflexes will divert blood away from the splanchnic system if there are any changes in the systemic blood flow that necessitate perfusion of vital organs. This hypothesis thus means that the resultant vasoconstriction could severely impair perfusion of the intestines.

The correlation between changes in intestinal circulation and intestinal injury has been under investigation for more than four decades. The first accepted hypothesis was that perinatal asphyxia leads to decreased intestinal blood supply, due to the extrinsic neurogenic blood flow redistribution. This in turn led to intestinal mucosal ischemia. However, the fact that NEC very rarely develops in the first seven days of life in preterm neonates, disproves this hypothesis.[15]

Also of note is the fact that sustained adrenergic stimulation has not been proven to cause any sustained intestinal hypoxia.[24]

Other causes of neonatal hypoxic stress have also been scrutinized. The following list includes other conditions or interventions that could lead to hypoxic hypoperfusion, and potentially lead to the development of NEC, although none of them have been conclusively proven as a sole cause for NEC:

- Sepsis
- Respiratory distress syndrome
- Cyanotic congenital heart disease
- Umbilical vessel catheterization
- Patent ductus arteriosus
- Polycythaemia[25]

The intestinal blood supply flows from the serosa on the outside to the mucosa on the inside. Thus, the first area where ischemia will become apparent during decreased perfusion, is in the mucosal layer.[7] This accounts for the fact that ischemia and necrosis is first visible in the mucosal layer, and then spreads to eventually involve the entire intestinal wall.

The last thing to consider when looking at hypoxic stress in neonates are vasoactive substances in the intestinal microcirculation. Multiple vasodilators and vasoconstrictors play an important role in balancing blood flow to the intestine. One of these important vasoconstrictors is Endothelin-1, which during pathological states can lead to ischemia in the intestinal wall.[7]

The role of intestinal microcirculation in the ischemic changes seen in NEC is well known, but it is still unclear whether the ischemia is the cause of the NEC, or rather a consequence of the disease process.

2.3.2 Changing intestinal microbiota

Most VLBW neonates are cared for in an ICU or high care set-up for the first few days to weeks of their life. This contrasts with healthy term neonates who are immediately handed over to the care of their mothers, where breastfeeding is encouraged. Close contact between mother and baby promotes the transfer of intestinal microflora, which is thought to improve nutrition and strengthen the intestine's epithelial barrier.[26] The normal microbiota is important in the immunity of the gut. By stimulation of the Paneth cells to secrete peptides, they promote an environment that resists the growth of pathogenic organisms.[27]

Preterm, VLBW neonates frequently end up being formula fed due to the mothers often having difficulty expressing their breastmilk, and the challenge of producing adequate quantities of breastmilk without the direct stimulation of the baby. Other risk factors for abnormal gut flora includes hygiene practices in the units, unnecessary and prolonged use of antibiotics, stasis of bowel content or reduced bowel motility, prolonged parenteral nutrition, and birth via caesarean section.[26] The dysbiosis of neonates delivered via caesarean, and the

importance there-of, section has been an ongoing dispute for the past decade. Even though some studies have proven that neonates born via caesarean section had a different intestinal microbiome from babies delivered via normal vaginal delivery (NVD), the importance clinical significance of these findings are still uncertain, and thus we did not make use of this parameter in our study.

Neonates who are cared for in ICU and High Care units are also more frequently exposed to nosocomial pathogens that are proven to be more resistant and virulent in their effects. These pathogens are thought to be the cause of Late Onset Neonatal Sepsis (LOS) and NEC in VLBW neonates. This theory is supported by the fact that most preterm neonates only develop NEC after the first week of life, as well as by the outbreak of clusters of NEC in certain units.[7]

2.3.3 Feeding and mechanical injury

In a 2009 Case Control Study, Henderson et. al showed that the rate of increase in feeding volumes is one of the important modifiable risk factors for the development of NEC in VLBW neonates. None of the neonates they evaluated had developed NEC before enteral feeds were commenced, but the neonates who developed NEC had a significantly quicker advancement in feed rate than the control group who did not develop NEC. They also found a significantly lower incidence of NEC in the neonates who were exclusively breastfed, versus the neonate who were formula fed, or received mixed feeds.[28]

The mechanisms in which differences in volumes and rates of enteral feeds influence the development of NEC is however still not well understood. The leading theory is that it is due to disruption of the intestinal mucosal integrity, intestinal blood flow, and intestinal motility.[27] One of the differences between the intestine of term and pre-term neonates is thought to be the mucus layer in the intestine preventing bacterial translocation. This layer is thought to be underdeveloped in the premature intestinal tract with discontinuations which can lead to trauma of the intestinal mucosa and bacterial translocation.[26]

During the third trimester, the foetus ingests amniotic fluid, which is rich in nutrients and growth factors, leading to intestinal development. The newborn infant then ingests breastmilk, which is also advantageous for intestinal development due to its complex composition of nutrients, immunomodulators, and hormones. This combination of amniotic fluid and breastmilk ingestion is thought to be the link to the low incidence of NEC in term breast-fed infants, whereas VLBW preterm neonates are born without their intestinal barrier having matured properly, rendering breastfeeding even more important in these infants.[29] However, even in breastfed VLBW neonates it is often impossible to advance their enteral feeds enough to achieve adequate levels of defence, and this has led to the addition of other substances such as probiotics, prebiotics and lactoferrin to promote intestinal immunity.[26]

A recent study by Harutuynyan et al compared neonates who received a multi-modal three-component enteral medication regime upon diagnosis of NEC, with neonates who did not. The medication included an antibiotic, an antifungal agent, and a probiotic. They found that the neonates who had received these enteral medications had a better outcome regarding mortality and the need for surgical intervention.[30] Nevertheless, as the data they used was only observational, this study could only generate a hypothesis, and further studies are indicated.

2.3.4 Immature intestinal barrier response

As discussed above, the intestine of a preterm neonate basically has the same make-up and qualities as the intestines of a foetus. This intestine is not yet adequately mature to deal with the same level of exposure and functionality as the intestinal tract of a term neonate. The mature intestine has both microbiota and physical barriers protecting it from bacterial translocation.[31]

The unique anatomical make-up of the intestinal barrier, a single epithelial layer that is continuously in contact with intestinal content, serves to maximize nutritional absorption. However, this quality can lead to ease of bacterial uptake as well, and thus the intestine needs special physical barrier defence mechanisms to prevent this.

These physical barriers include intestinal mucus production, peristaltic movement, cell surface glycoconjugates, and tight junctions between intestinal epithelial cells.[31] All of these barriers serve to limit the amount of bacteria present in the gut lumen, as well as preventing attachment of these bacteria to the intestinal epithelium, which could lead to bacterial translocation.

In premature neonates these physical barriers are poorly developed. For instance, the first intestinal layer that gets into contact with bacteria is the mucous layer of the gut that overlies the epithelial cells. This mucous layer has been found to be less dense and to have lower levels of secretory immunoglobulins (see 2.3.5 Immature intestinal inflammatory response) in the premature gut.[31] Some studies have also suggested that there can be a paucity of mucin producing goblet cells in some genetically predisposed neonates.[32]

The next layer of the physical intestinal barrier includes the Paneth cells in the terminal ileum, and the Lieberkühn crypts which plays an important role in healing of the intestinal mucosa once damaged.[26] Both of these have been found to be immature, impairing their functionality in premature neonates. The combination of the defective immune defence mechanism, and inability to adequately heal vulnerable areas in the intestinal mucosa, can lead to bacterial translocation.[33]

Also, important to take note of in the pathophysiology of NEC in premature neonates, is the paucity of peristaltic activity. The gut motility of VLBW neonates is significantly lower than that of term neonates, leading to stasis of the bowel content. This leaves the immature intestinal mucosa in contact with bacteria as well as other potentially dangerous substances for prolonged periods of time, potentially leading to damage and bacterial overgrowth and translocation.[34]

2.3.5 Immature intestinal inflammatory response

In addition to the physical barriers, there is also a functional barrier that is supposed to limit the growth of those bacteria which manage to overcome the physical barriers. This consists of the immunological factors such as intestinal lymphocytes, salivary IgA, and biochemical factors such as gastric acid secretion and proteolytic enzymes. Neonatal levels of these bioactive substances are decreased, further leading to an increased risk of bacterial translocation and the subsequent development of NEC.[31]

The most important inflammatory mediators identified that is known to play a role in intestinal maturity are:

- Platelet activating factor (PAF)
- Tumour necrosis factor (TNF)
- Interleukins (1, 3, 6 and 8)
- Epidermal growth factor
- Nitric oxide

PAF is a phospholipid inter- and intra-cellular inflammatory mediator that causes intestinal epithelial cell apoptosis, but the exact mechanism of this intestinal injury is still under investigation. Patient's with inflammatory bowel disease has been shown to have elevated serum and intestinal PAF, and in patients with NEC the PAF levels have correlated with disease severity.[31] In 2001 Rabinowitz et al found increased PAF levels in neonates several days before NEC became clinically evident. This can indicate that the raised serum PAF is an important initiating factor in the development of NEC.[35] Further support for this theory is the reduced incidence of NEC found in rats who received PAF-degrading enzyme PAF acetylhydrolase.[36] Other than the direct inflammatory effect of raised PAF levels, it also initiates the increased production of other inflammatory mediators such as TNF, prostaglandins, complement and thromboxane.[37]

This inflammatory cascade, which is initially triggered by cell damage and apoptosis, will eventually lead to intestinal necrosis if uninhibited. Intestinal enterocytes keep intestinal inflammatory responsiveness under control. In premature neonates the enterocytes have not adequately matured yet when the intestinal epithelium is exposed to exogenous bacteria, and this can lead to an exaggerated inflammatory response. Compared to adult intestinal epithelial cells, foetal intestinal epithelial cells produce much more interleukin 8 (IL-8). IL-8 is a chemoattractant cytokine that draws neutrophils into tissues, activate them, and alter their adhesion molecules.[31]

2.4 Risk factors

Although no exact cause for NEC could be identified yet, multiple risk factors have been identified that predisposes neonates to the development thereof. But even identifying these risk factors is only one step in potentially preventing the development of NEC.

2.4.1 Prematurity and intrauterine growth restriction

Even though NEC also rarely occurs in term neonates, the vast majority of neonates who develop NEC are premature, and of low birth weight. Term neonates also tend to develop NEC earlier than premature have more clearly defined associated risk factors such as low Apgar scores, congenital heart disease, and sepsis. Intra uterine growth restriction (IUGR) in term neonates also poses a significant risk.[38] This is due to the foetal circulation redirecting blood away from the mesenteric system, towards more vital organs such as the brain, heart and kidneys.[11] Regarding birth weight, the risk of developing NEC rises with decrease in birth weight.[39]

2.4.2 Neonatal hypoxia

The first few days in a neonate's life is critical. Any instability in perfusion to the bowel during this period could increase the risk for development of NEC. Tissue perfusion is dependent on the oxygen delivery to tissue (D02), and the oxygen consumption of that tissue (VO2).[40]

$$DO2 = CaO2 \times CO$$

$$VO2 = CO \times (CaO2 - CvO2)$$

Thus, the most important physiological parameters that determine oxygen delivery and consumption at tissue level are the oxygen content of arterial blood (CaO2), and the cardiac output (CO). Any changes in these two parameters could negatively impact the tissue perfusion to the intestines.[40]

$$CaO2 = (1.34 \times (Hb) \times SpO2) + (0.003 \times PaO2)$$

This equation shows us that the oxygen content of blood is dependent on the patient's arterial oxygen saturation, and haemoglobin (Hb) concentration (thus oxygen carrying capacity). Any drop in oxygen saturation due to hypoxia would lead to a drop in the oxygen content of the blood. Due to shunting mechanisms redirecting blood to the more vital organs, the tissue perfusion to less vital organs such as the intestines would drop, leading to poor perfusion in the mesenteric beds.[40]

Conditions or interventions that could cause hypoxia in neonates include the following:

- Recurrent apnoea
- Respiratory distress syndrome
- Umbilical artery catheterisation
- Birth asphyxia
- Maternal cocaine use
- Exchange transfusions[11]

2.4.3 Formula feeding

As discussed previously in the pathophysiology section (See 2.3.3 Feeding and mechanical injury) timing, volume and type of feeds has been shown to be important variables in the development of NEC. The most important variable that has been proven to be of clinical importance is the difference between exclusive breastfeeding, and formula feeds.[38] Exclusively formula fed neonates have been reported to be three times more likely to develop NEC than their breastfed counterparts.[11]

The important differences between breast and formula feeds implicated in this increased risk are as follows:

- Different probiotic make-up
- Higher protein content in breastmilk
- Higher caloric density in breastmilk

- Higher osmolality in formula milk
- Higher levels of free fatty acids in formula milk
- Lack of maternal protective factors in formula milk[11]

The fact that breastfeeding is proven protective against the development of NEC, especially in preterm neonates, is an easy, inexpensive, practical way of eradicating an important risk factor for NEC. Especially in lower income countries.

2.4.4 Antenatal steroids

Antenatal steroids in the form of dexamethasone or betamethasone, are given to mothers who go into preterm labour between 24 and 34weeks, to assist in foetal lung maturation. If the steroids are given and delivery can be delayed to 24hours after the first dose, deaths from neonatal respiratory distress have been shown to significantly decrease.[41]

Various studies have been done implicating antenatal steroids as both a risk factor and a protective factor in the development of NEC. However, all the studies agree that potential benefit gained from the respiratory effects of antenatal steroids, out way the risk of development of NEC.

2.4.5 Congenital heart disease

As discussed in the section on neonatal hypoxia as a risk factor (2.4.2 Neonatal hypoxia) cardiac output (CO) has an important influence on tissue oxygen perfusion.

$$CO = HR \times SV$$

The cardiac output is directly influenced by heartrate and stroke-volume.[40] Neonates with congenital heart disease, specifically those with a left-to-right shunt, such as patent ductus arteriosus, decrease the stroke-volume, and since neonates already have a high baseline heartrate, they have a limited capacity to compensate by increasing their heartrate. Decrease cardiac output will lead to decreased tissue oxygen delivery.[11]

2.5 Diagnosis

2.5.1 Clinical

Recognition of the clinical signs of NEC is of great importance to any doctor working with neonates, due to the possibility of rapid and fatal progression of this disease. Early signs are very non-specific, and can include any or all the following:

- Apnoea
- Changes in baseline heartrate
- Changes in level of consciousness or irritability
- Temperature instability

Of these early clinical markers, changes in heartrate has been studied. In 2013 Utone et al demonstrated that increased heart rate variability could be identified up to 18hours prior to NEC

being diagnosed in neonates in an intensive care unit. By continuous heart rate monitoring, they defined increased variability by increases in decelerations, and decreases in accelerations.[42] More recently, a study conducted at the University of Pretoria in South Africa, demonstrated a persistent increase in heart rate 72hours before neonates with confirmed NEC deteriorated up to the point of needing surgical intervention.[43] Both of these studies indicate the importance of changes in heartrate as an early, and valuable identification tool in neonates who both develop NEC, and subsequently deteriorate.

Signs which implicate the gastro-intestinal tract specifically include:

- Feeding intolerance
- Increased gastric aspirates
- Bilious/bloody vomiting
- Abdominal distention
- Blood per rectum

Later, abdominal tenderness, discoloration of the abdominal wall, and a palpable abdominal mass, will indicate progression of the disease process.[16] Recurrent, single observer examination of the abdomen is necessary to pick up any deterioration which might indicate progression to bowel necrosis.

An important distinction is also made between the presentation and onset of symptoms in preterm neonates, as opposed to term neonates who develop NEC. Preterm neonates tend to develop NEC after 15 – 20days of life, whereas term neonates develop NEC within the first few days of life.[7]

2.5.2 Radiological

To confirm the presence of NEC, radiographic evidence is however required. Although clinically suspected, the diagnosis can only be confirmed by certain signs on plain abdominal X-rays.

- Pneumatosis intestinalis
- Adynamic ileus
- Air in the portal venous system (branching radiolucency extending to within 2cm beneath the liver capsule)

The amount of non-pathological intra-mural gas that can be detected may vary with gestational age. Most term neonates will exhibit some evidence thereof on plain abdominal films, while the presence and possible identification of pneumatosis in preterm neonates may be impaired.[44]

Another imaging modality that can be utilized in the diagnosis and management of NEC is abdominal ultrasonography. The additional use of colour duplex Doppler imaging can assist in evaluating the thickness of the bowel wall, the echogenicity thereof, and appraising the quality of bowel wall perfusion, as well as identifying fluid collections, peristalsis, and pneumatosis too small to be visible on plain films.[16]

In some hospitals, abdominal ultrasonography can be of use to evaluate the presence of necrotic bowel; however, the limitations thereof include inter-observer variability, oversensitivity for detecting abnormalities, and limited availability in all settings.

Imaging modalities that have not proven of practical value in the diagnosis and evaluation of NEC include:[7]

- Contrast screening studies
- Computed Tomography
- Magnetic Resonance Imaging

2.5.3 Laboratory studies

Although very non-specific in making the diagnosis of NEC, biochemical and haematological evaluation can assist in the management of the patient, and identification of the presence of bowel necrosis. Common findings can include:

- Neutropenia or neutrophilia
- Thrombocytopenia
- Metabolic acidosis (persistent)
- Hypo- or hyperglycaemia
- Electrolyte disturbances (particularly hyperkalaemia and hyponatremia)

A combination of clinical and radiological evidence makes scoring the severity of the insult possible, by making use of the Modified Bell's Staging (Table 1).[1] Laboratory studies usually reveal a nonspecific inflammatory process. Currently there is no specific test available to confirm NEC or predict the course thereof.

STAGE	CLINICAL FINDING	RADIOGRAPHIC FINDINGS	GASTROINTESTINAL FINDINGS
I: Suspected A	Apnoea Bradycardia Temperature instability	Suggestive of ileus only	Increased NG aspirates Occult Blood in stool Mild abdominal distention
B	Same as IA	Same as IA	Same as IA, plus gross blood in stool
II: Definite A – Mildly ill	Apnoea Bradycardia Temperature instability	Ileus with dilated loops Focal pneumatosis	Macroscopic blood in stool Severe abdominal distention Absent bowel sounds
B – Moderately ill	Thrombocytopenia Mild metabolic Acidosis	Widespread pneumatosis Ascites	Abdominal wall oedema Tenderness

		Portal Venous Gas	Palpable bowel
III: Advanced A – Severely ill, bowel intact	Mixed acidosis Oliguria Hypotension Coagulopathy	Prominent bowel loops Worsening ascites No free air	Worsening wall oedema Erythema Induration
B – Severely ill, bowel perforated	Shock Deteriorating Laboratory values and Vital Signs	Pneumoperitoneum	Perforated bowel

Table 1: Modified Bell's Staging for Necrotising Enterocolitis [1]

2.6 Differential diagnosis

From the previous section, it should be clear to the reader that the diagnosis of NEC is difficult to make due to the non-specific presenting symptoms. Especially in the early stages, a few other disease processes found in neonates could mimic the symptoms of NEC.

- Hirschsprung's disease (with or without Hirschsprung's Associated Enterocolitis)
- Ileus due to sepsis
- Malrotation with midgut volvulus
- Meconium ileus
- Small bowel atresia (especially Type 0)
- Spontaneous intestinal perforation
- Gastric perforation
- Maternal ingested blood
- Food protein-induced allergies [45]

All these conditions need to be investigated and actively excluded. Malrotation with midgut volvulus is a surgical emergency that can have devastating consequences if the diagnosis is missed.[1] Spontaneous intestinal perforation, also known as focal intestinal perforation, can have a very similar clinical presentation as NEC Grade IIIB, and essentially the management always requires surgical intervention, whether by the insertion of peritoneal drains, or laparotomy and resection of the perforated segment.[46]

2.7 Prevention

Previously the focus of neonatologists was solely on the management of NEC once it has already developed. This unfortunately did not lead to any significant improvement in the outcomes or long-term complications. Currently the focus of research has moved more towards the prevention of the development of NEC in susceptible neonates. Thus far, the main focus in prevention of NEC has been adjustments in feeding strategies and modifying the gastro-intestinal flora.

Where feeds are concerned, the time of initiation of feeds, type of feeds (breastmilk versus formula feeds), fortification of feeds, and rate of escalation of feeds, are important variables. Initiating early feeds in preterm neonates has been thought to increase the risk for developing NEC, although a systemic review by Kennedy and Tyson in 2000 concluded that the available studies had a too small sample size to adequately back this finding.[47] Neonates who are breastfed have been found to have a lower risk of developing NEC than those who receive formula feeds, independent of the age at initiation of feeds.[48,49] Although some studies have suggested that a higher rate of advancement of feeds once initiated carries a higher risk for developing NEC,[50] the results from these studies are still being scrutinized. Current feeding strategies at the NICU's in PTH and UAH include delaying feeds in preterm neonates, slower rate of feed increase in preterm neonates, and advocating for breastmilk feeds rather than formula feeds whenever possible.

In addition to changes in feeding strategies, the use of enteral antibiotics and probiotics has been suggested to protect against NEC,[44] but this is still controversial and is not currently being utilized at any of our local units. (See pathophysiology)

2.8 Management

The management of NEC depends on the clinical condition of the neonate, with surgical intervention only being utilized once necrosis or subsequent perforation of the bowel is present. Initial treatment is aimed at general patient support, as well as finding and reversing the causative factor, and managing the bacterial component with antibiotics. The most important part of the management of neonates with NEC is deciding who needs surgical intervention, and the timing thereof. Once a patient has been diagnosed with NEC, they need to be very closely monitored to pick up any detrimental changes timeously, and act upon them.[7]

2.8.1 Medical management

A septic work-up is done on all neonates showing signs of NEC, after which empirical antibiotic therapy will be commenced. All enteral feeds are discontinued to minimize the risk of further damage to the diseased intestine, and a nasogastric tube (NGT) is inserted to decompress the stomach and proximal bowel. Parenteral feeds to optimize nutrition, and intravenous fluid therapy is also started.[22]

All neonates with clinical signs and symptoms suggestive of NEC need to be closely monitored with serial blood-gas analysis, abdominal examination, and abdominal X-rays at least every 6 – 8hours initially to quickly pick up any deterioration in the early stages of the disease.[51]

Grade I and II disease can mostly be managed conservatively, with clinical improvement expected within 48 - 72 hours, after which feeds can be restarted and increased as tolerated.[7] Lack of clinical improvement, or deterioration in clinical state should prompt involvement of the surgical team, and possible re-evaluation of the diagnosis.

2.8.2 Surgical management

Some controversy still exists regarding the exact timing of surgical intervention, but the most commonly utilized clinical parameters include bowel perforation (distinguished by pneumoperitoneum on abdominal X-rays, or the finding of bowel content on paracentesis).[1] Absolute indications for surgery include:

- Palpable abdominal mass (suggestive of necrotic bowel with sealed off perforation)
- Clinical deterioration despite maximum medical intervention
- Evidence of intestinal obstruction on plain abdominal film
- Evidence of adynamic segment of bowel on plan abdominal film (suggestive of necrotic bowel)
- Worsening biochemical picture

Relative indications for surgery include:

- Increased abdominal tenderness, distention, or discolouration
- Portal vein gas
- Pneumoperitoneum
- Positive paracentesis
- Severe thrombocytopenia.

Patient with severe NEC Grade IIIB may be extremely ill and may require aggressive resuscitation before and during surgery. Blood products, inotropic support, and ventilatory support need to be available immediately.

Surgical intervention ranges from primary peritoneal drainage, to laparotomy and resection of necrotic bowel. Peritoneal drainage can be used in neonates who have developed respiratory compromise due to extensive pneumoperitoneum or ascites, in an attempt to stabilize them for transfer to theatre. It has also proven valuable as a primary intervention for Grade IIIB NEC in some units, although there are studies that have disproven its use as a sole therapeutic intervention.[7,52]

The goal or surgery is to remove the necrotic bowel, whilst aiming to leave enough viable small bowel to prevent the development of short bowel syndrome. Depending on the clinical condition of the patient during surgery, the surgeon might decide to resect the necrotic bowel and do a primary anastomosis in a very stable patient with normal clinical and biochemical parameters, resect the necrotic bowel and bring out stomas if the bowel is in poor condition or the patient's condition is not favourable, or do a clip-and-drop surgery with the aim of definitive surgery later when the patient is more stable.[7,18,53] All options have been reported and are acceptable, and it remains at the discretion of the surgeon to decide what would be the best option for their patient.

Laparoscopy may be useful in confirming the presence of necrotic bowel or sealed of perforations, but once identified the recommendation is that the surgery be converted to an open laparotomy.[54]

2.9 Transfusion Associated NEC (TANEC)

2.9.1 Definition

Transfusion Associated Necrotising Enterocolitis (TANEC, referred to as TRANEC by some authors) refers to NEC that develops in neonates within 48-hours after receiving an RBC transfusion.[55] A study in Canada in 2013, by Stritzke et al, showed that neonates with a lower mean gestational age and lower mean birth weight were at higher risk for developing NEC within two days after receiving an RBC transfusion. However, they did not identify any difference in morbidity or mortality between the patients who had developed TANEC, and the neonates who had developed NEC without receiving an RBC transfusion.[56]

The association between transfusion of blood products and the development of NEC in preterm neonates was first described in 1987 by McGrady et al,[57] although very little further research was conducted into this phenomenon until the last decade. This has led to larger neonatal centres reviewing their transfusion strategies, as well as adopting protocols where at risk neonates do not receive enteral feeds immediately before and after a transfusion of blood products. There is however still wide spread controversy regarding this topic, with some studies showing no significant correlation between transfusion of RBCs and the development of NEC.[58]

2.9.2 Pathophysiology

TANEC, also called transfusion related acute gut injury (TRAGI) in some units, is thought to be an adverse reaction to blood transfusions, similar to the pulmonary counterpart found in adults, namely "transfusion related acute lung injury" (TRALI).[59] This pathological process is hypothesized to be due to a multifactorial insult on the intestinal barrier. Firstly, at cellular level, there is thought to be an immunological response to Human Leucocyte Antigen (HLA), and other antigens present in the transfused blood. This leads to a cascade of endothelial cell activation.[3] This immune reaction, in combination with the increased viscosity of the blood products, is thought to lead to a decrease in mesenteric blood flow directly after transfusions. Along with this, enteral feeds in the presence of decreased mesenteric blood flow can lead to the development of NEC.[5] The last factor influencing the perfusion state of the bowel after a blood transfusion, can be the age of the donor blood, leading to a decrease in capacity to deliver oxygen to tissues, and subsequently to vasoconstriction and ischemia.[60]

2.9.3 Transfusion guidelines

The neonatal population receive some of the highest amounts of blood transfusions in hospitals. However, very few guidelines exist regarding the threshold for transfusions in neonatal units. Even where guidelines do exist, it is seldom adhered to. This is mostly due to the previous school of thought being that to optimize the oxygen delivery to tissues, the haemoglobin delivery system should be optimized by transfusing packed cells. However, recent studies have shown that unnecessary transfusions of RBCs have more detrimental effects in the neonatal population than previously thought. Also that oxygen delivery to peripheral tissues may be impaired at too high an artificial haematocrit.[61]

Three studies done between 2006 and 2011 demonstrated that up to a third of neonates who had developed NEC, had received RBC transfusions within 48-hours prior to the development of the NEC.[62,63,64] However, some controversy still exist regarding whether it is the RBC transfusion that predisposes neonates to the development of NEC, or the pre-existing severe anaemia. In 2016 Patel et al conducted a observational cohort study, evaluating the these two entities, and identified that the severe anaemia, rather than the RBC transfusion was the bigger risk factor for the development of NEC.[65]

This possible existence of TANEC has led to more scrutiny in the practice of RBC transfusions. In 2009 Valieva et al published a study evaluating the previous University of Washington Neonatal Intensive Care Unit 2006 Transfusion Guidelines,[66] and found that no clinical benefit to giving RBC transfusions above the limits of their new clinical guidelines (Table 2) could be identified. They found that more neonates needed respiratory support after transfusion and identified a positive link between RBC transfusions and Bronchopulmonary Dysplasia in neonates. They also identified a possible association between RBC transfusions and the development of NEC, and as a result of their study, the guidelines were amended to allow for lower haematocrit levels in neonates, as well as a more clinical approach to deciding whether or not to transfuse a patient.[61]

The most commonly used guideline for RBC transfusion in neonates, are as set out in Table 2.[61]

Indications for Transfusion of RBC's in Neonates	
HCT	AGE and CLINICAL CONDITION
<35%	≤1week of Life **AND** Unstable*
<28%	≤1week of Life **OR** Unstable*
<20%	>1week of Life **AND** Unstable*
*Unstable clinical condition is defined as a state of poor oxygen delivery, e.g. Respiratory distress/apnoea, Cardiovascular deterioration etc.	

Table 2 : Indications for RBC transfusions in neonates [61]

2.9.4 Feeding during transfusion

As mentioned before, the impact of enteral feeds on mesenteric blood flow and oxygen delivery during RBC transfusions, is of great importance. A study conducted by Krimmel et al in 2009 found that there was decreased flow in the Superior Mesenteric Artery (SMA) postprandially following an RBC transfusion, suggesting an increased risk for intestinal ischaemia.[67] Wan-Huen et al did a similar study in 2013 which found that neonates who were fed within two days before receiving an RBC transfusion were eight times more likely to develop TANEC.[60] By making use of near infrared spectroscopy in measuring the mesenteric regional oxygen saturation in neonates receiving transfusions, Marin et al showed a decrease in mesenteric oxygenation in patients receiving concurrent feeds, as opposed to an increase in patients in whom enteral feeds were halted during the transfusion. They also found that more premature neonates were increasingly vulnerable to changes in mesenteric blood flow after RBC transfusions, and subsequent mesenteric ischaemia.[5] This study created a physiological base for the findings in the previous studies.

In contrast, the FEEDUR RCT trial conducted in Australia between 2016 and 2017, proved no difference in splanchnic oxygen delivery in infants that received enteral feeds during RBC transfusions. They measured the splanchnic-cerebral oxygen ration (SCOR) and mean splanchnic fractional oxygen extraction (FOE), before during and after RBC transfusion in 60 neonates. Also of note, none of the neonates included in this study developed NEC, but the incidence of NEC in VLBW neonates in the hospital in question is significantly lower that the international standard, and this needs to be taken into account, along with the small sample size of the study.[68]

The first definite guideline for keeping neonates NPO peri-transfusion, was the Duke Intensive Care Nursery protocol first implemented there in February of 2009. This states that all enteral feeds and fluids should be withheld for four hours before, during, and after the RBC transfusion. A study done by DeRienzo et al in 2014 monitoring the effect of feeding practices in the development of TANEC, made use of this protocol to evaluate their findings.[69] They saw a decrease in the incidence of NEC in their units following improved transfusion practices, although a clear link between the change in feeding protocol and the development of TANEC could not be proven due limitations in their study.

Lastly of note is the different feeds that can be administered to VLBW neonates. The protective effects of breast milk feeds in VLBW neonates has been well established, and its importance in the prevention of NEC cannot be overstated. In a Cochrane review in 2008, eight studies that evaluate the effect of breastmilk on various areas of neonatal health and development were compared. They found that formula feeds had a 2.5% relative higher risk for developing NEC, compared to donor milk.[70] There are currently no studies comparing breastmilk versus donor milk in the development of TANEC.

2.9.5 Exchange transfusion

Indications for exchange transfusions in neonates include:[71,72,73]

- Severe hyperbilirubinemia due to haemolysis:
    - Maternal blood incompatibility
    - Enzymopathies
    - Membranopathies
- Severe Hyperkalaemia
- Gestational alloimmune liver disease (GALD)
- Severe sepsis
- Disseminated intravascular coagulopathy (DIC)

The guidelines regarding exchange transfusion in neonates are better researched and established. Exchange transfusions have been identified as an independent risk factor for the development of NEC.[25]

2.10 Prognosis

Since the first description of NEC in the 1980's there has been extensive research done into the cause and treatment thereof. Significant advances in neonatal care in the past two decades have led to improved survival for premature infants, but the mortality rate for neonates with NEC have stayed the same at between 15% and 30%.[13,14,74] The mortality rate for neonates with NEC who require surgical intervention is much higher (up to 50%), but has decreased slightly over the past few decades due to improved diagnostic, anaesthetic, and surgical skills. This can be attributed to the fact that once they reach the disease severity where they require surgical intervention, neonates tend to be clinically unstable, and have developed failure of other organ systems. The

risk that the high mortality rate is due to late identification of the need for surgery needs to be kept in mind though, to help prevent this complication.[7,74]

In 2006, Kessler et al developed a presurgical mortality scoring system for neonates with NEC.

	0	1	2
Gestational age	>32 weeks	32 – 30 weeks	<30 weeks
Bell's Stage	I	II	III
Platelets (10^9/L)	>200	200 – 150	<150
Lactate (mg/dL)	<2.5	2.5 – 3.3	>3.3

Table 3: Presurgical NEC Mortality Score[75]

By using four variables, the gestational age, Bell's Stage, serum platelet count, and serum lactate level, a mortality risk can be calculated. Increased prematurity, higher Bell's staging, thrombocytopenia, and lacticaemia, leads to an increased risk of mortality. A score of between 1 and 3 had a 0% mortality rate, a score of between 4 and 6 had a 20 – 30% mortality rate, a score of 7 had a 60% mortality rate, and a score of 8 carried a 100% mortality rate. The predictive sensitivity of this scoring system was 0.72, with a specificity of 0.72.[75]

2.11 Complications and long-term sequelae

Other than the high mortality rate associated with NEC, neonates who develop and are successfully treated for NEC also face a barrage of short- and long-term complications, including but not limited to the following:

- Recurrence of NEC
- Sepsis
- DIC
- Shock
- Multi-organ dysfunction and failure
- Intestinal obstruction due to strictures
- Short bowel syndrome
- Entero-cutaneous fistulae
- Entero-colonic fistulae
- Impaired neurological outcome [7,2]

Regarding short bowel syndrome: not only neonates who were managed surgically and had bowel resections are at risk, but also the ones who were managed conservatively. This is due to impaired absorptive capacity of the diseased bowel. Up to 42% of neonates who required surgical intervention for NEC will subsequently develop short bowel syndrome.[7]

Premature neonates are at increased risk for neurodevelopmental delays due to the interruption of their intra-uterine development. NEC and sepsis added on to this already significant risk, can lead to devastating long-term neurological complications. Reese et al. reported that up to 45% of neonates who survive NEC have a poor neurodevelopmental prognosis, with the risk being even higher for neonates who received surgical intervention. These included neurodevelopmental problems such as deafness, cerebral palsy, visual impairment, mental developmental impairment, and psychomotor impairment.[76]

2.12 Conclusion

There is no question about the importance of further insight and research into NEC being of vital importance to the preterm neonatal population. NEC is a common, life-threatening disease with an increased incidence seen due to improvements in neonatal care. The burden NEC places on health care systems, hospitals, neonatal personal, as well as society due to the long-term complications, cannot be ignored.

RBC transfusions are not without consequences, despite their often lifesaving impact. It is important that the decision to give a VLBW neonate an RBC transfusion, always take all the potential risks and benefits into account. To aid in this decision making, protocols and guidelines need to be in place.

Currently very little is known regarding the exact aetiology of this devastating condition, and our focus has only recently started shifting away from management of NEC once it has developed to finding causes and risk factors and attempting to prevent the condition. Since the mindset is changing, the focus needs to shift to finding possible causes and doing adequate research to enable evidence based clinical practice.

Chapter 3: Problem Statement

3.1 Problem statement

Is there an association between RBC transfusions in VLBW neonates, and the development of NEC? If so, is there an association with the severity of the ensuing NEC?

3.2 Aims and objectives

The aim of this study is to evaluate the RBC transfusion practices in VLBW neonates in two Academic Neonatal Units in Bloemfontein, Free State, and to determine whether there is an association between transfusion practices and the development of NEC.

3.2.1 Primary objectives

The primary objectives are:

- Comparing and describing the RBC transfusion practices (the haematocrit and clinical condition leading to RCC transfusion) between the two hospitals
- Recording any changes in RBC transfusion practices over a 5-year period
- Evaluating possible differences in outcomes after RCC transfusion, specifically pertaining to the development of NEC

3.2.2 Secondary objectives

- The haematological indications for RBC transfusions
- The clinical state of neonates during RBC transfusions
- Whether or not they were receiving enteral feeds during the RBC transfusion
- The possible association between RBC transfusions, the timing of enteral feed in relation to the RBC transfusions, and the development of NEC, including its severity

Evaluation of the RBC transfusion practices in association with the development of NEC will aid us in determining whether an association exists between RBC transfusions in neonates, the time frame of enteral feeds in relation to the transfusions, and the development of NEC, as well as the severity of the NEC developed. By evaluating these practices over a 5-year period and being able to identify possible shortcomings in the RBC transfusion protocols, we hope to significantly improve our clinical practice.

Chapter 4: Research Design

4.1 Introduction

This chapter serves as an explanation of the data collected by the principle investigator, the data form used to capture the data, and the statistical methods used to interpret the data.

4.2 Pilot Study

A brief pilot study was conducted to evaluate the feasibility of data collection, and the adequacy of the data form used to document the study parameters. This pilot study included 20 patients and showed us that the initial plan to make use of the statistics of the Department of Paediatrics to capture patients, would not be possible due gaps in their statistics. A new method to locate VLBW neonates who were treated at UAH and PTH during our study period was then devised, by using the online statistics captured by the online Meditech© system. This new method proved accurate, although significantly more time consuming, and the study could move forward, albeit with a new adjusted timeline. Since no changes were made to the study protocol or data collection sheet after completion of the pilot study, the 20 patients whose data was collected in the pilot study, were also included in the final study data.

4.3 Basic data collection

The study is an analytical retrospective review.

This study was conducted using data collected from the Neonatal Units of Universitas Academic Hospital, and Pelonomi Tertiary Hospital Bloemfontein, South Africa.

Patient inclusion criteria:

> All Very Low Birth Weight (1000g – 1499g) neonates born in the Pelonomi Tertiary Hospital, and Universitas Academic Hospital, and/or admitted to the respective neonatal units in a 5-year period. The time frame for data collected was between January 2012 and December 2017.

Patient exclusion criteria:

- Neonates born in other hospitals, and subsequently admitted after 48-hours of life
- Any other significant congenital abnormalities
- Neonates who demised within 72 hours of life

Data was collected by obtaining the Paediatric Departmental admission statistics from Meditech©. All VLBW neonates were identified. The electronic notes of all the neonates were reviewed, and neonates who had received RBC transfusions were identified. By reviewing these patients blood results on the with the NHLS-Labtak database, the pretransfusion and post transfusion haematocrits were recorded. The date of the RBC transfusion, as well as the pre-

transfusion and post-transfusion haematocrit, and the pre-transfusion clinical condition of the patient was recorded.

Furthermore, patients within the VLBW group who developed NEC were identified. It was recorded whether they developed the NEC before an RBC transfusion, or within 48-hours following one.

Principle data collected consisted of the following parameters:

- Date of birth
- Birth weight
- Gestational age
- RBC transfusion related data
    - Date of transfusion
    - Pre-transfusion haematocrit
    - Post-transfusion haematocrit
    - NPO during transfusion (Yes / No)
    - Clinical condition during transfusion
    - Ventilatory support during transfusion
- NEC related data
    - NEC developed (No / Within 48-hours after RBC transfusion / Before transfusion)
    - Severity of NEC (Modified Bell's Staging)

4.4 Statistical analysis

The data for PTH and UAH patients was captured and analysed separately, to enable a comparison between the two hospitals. The data analysis was done by the Department of Biostatistics at the University of the Free State. Results were summarised by frequencies and percentages (categorical variables) and means, standard deviations or percentiles (numerical variables). Associations were assessed by relative risks with 95% confidence intervals, and appropriate hypothesis testing.

4.5 Ethics

Approval to conduct the study was obtained from the Health Sciences Research Ethics Committee of the University of the Free State, as well as the Free State Department of Health. Patient confidentiality was always respected by assigning patients individual study numbers on data sheets and keeping data on a secure password protected computer. All data will be kept for 15years after conclusion of the study.

HSREC Approval Number: UFS-HSD2017/0616

FSDoH Approval Number: FS_201805_003

Chapter 5: Results

5.1 Introduction

This chapter will discuss the following results obtained from this study.

- Epidemiological data
- RBC transfusion results
- NEC results

5.2 Epidemiological data

From 1 January 2012 till 31 December 2016, 1585 VLBW neonates were admitted and treated in the neonatal units at PTH and UAH. Of these, 1044 (66%) were admitted and treated at PTH, and 541 (34%) at UAH. Over the 5-year study period the number of VLBW neonates treated at each of these hospitals remained stable with no fluctuations in number. Thus, the number of VLBW neonates treated at these two units have remained more or less the same throughout the study period.

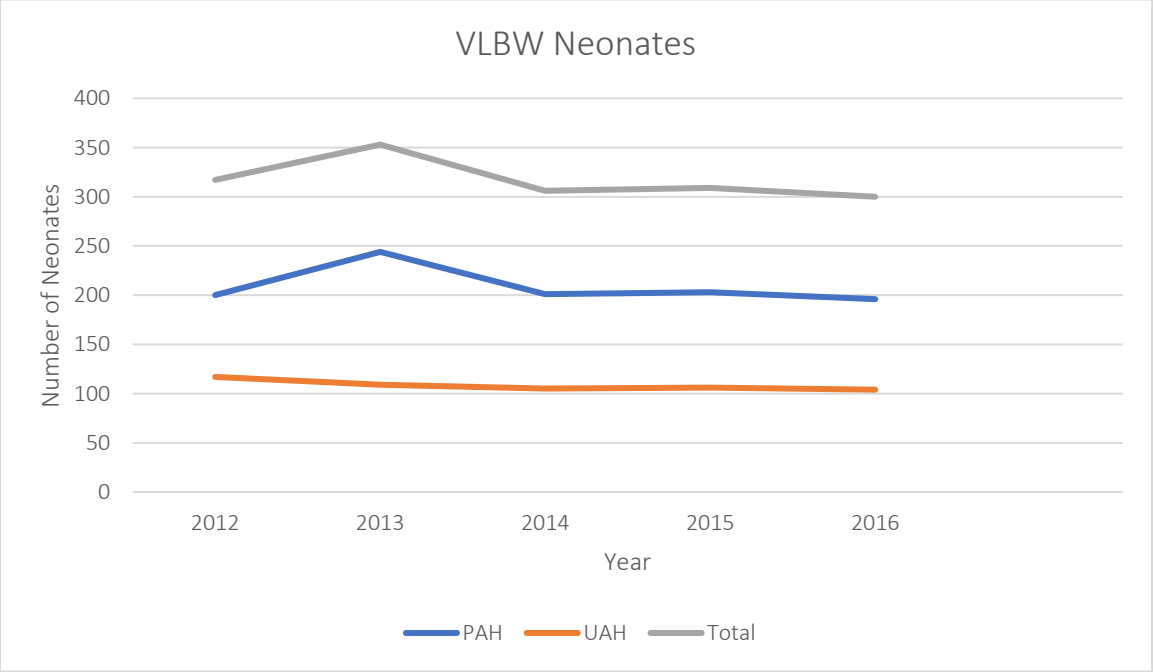

Figure 1: Number of Very Low Birth Weight neonates treated in Pelonomi Tertiary Hospital and Universitas Academic Hospital (2012 – 2016)

Before exclusion of the VLBW neonates who demised within 72-hours of life, together with those who suffered from other significant congenital abnormalities, a profile of the congenital abnormalities found in the VLBW neonates was determined.

At PTH, 63 of the neonates were excluded because they had demised within 72-hours of life, 18 were excluded due to insufficient data in their files, and 13 were excluded due to other significant congenital abnormalities. The data of 950 neonates from PTH (60% of the total number of VLBW neonates evaluated) were included in the study. The median birth weight of these patients was 1300g, with a median gestational age of 30 weeks.

At UAH, 35 of the neonates were excluded because they had demised within 72-hours of life, 9 were excluded due to insufficient data in their files, and 21 were excluded due to other significant congenital abnormalities. The data of 476 neonates from UAH (30% of the total number of VLBW neonates evaluated) were included in the study. The median birth weight of these patients was 1200g, with a median gestational age of 29 weeks.

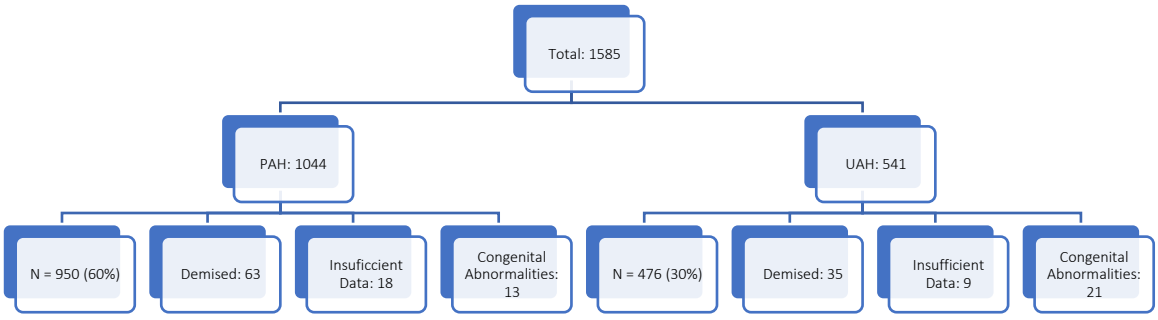

Figure 2: Profile of Very Low Birth Weight neonates excluded from the study (2012 – 2016)

The congenital abnormalities found in VLBW neonates excluded from the study, were as follows:

Congenital Abnormality			
Duodenal atresia	7	4,4: 1000	21%
Oesophageal atresia (±Tracheo-oesophageal fistula)	4	2,5: 1000	12%
Malrotation	3	1,9: 1000	9%
Myelomeningocele	3	1,9: 1000	9%
Trisomy 21 (Down syndrome)	3	1,9: 1000	9%
Hirschsprung's disease	2	1,3: 1000	6%
Anorectal malformations	2	1,3: 1000	6%
Gastroschisis	2	1,3: 1000	6%
Trisomy 18 (Edward syndrome)	2	1,3: 1000	6%
Small bowel atresia	1	0,6: 1000	3%
Omphalocele	1	0,6: 1000	3%
Meconium ileus	1	0,6: 1000	3%
Osteogenesis imperfecta	1	0,6: 1000	3%
Noonan syndrome	1	0,6: 1000	3%
Turner syndrome	1	0,6: 1000	3%

Table 4: Profile of congenital abnormalities in Very Low Birth Weight neonates in Bloemfontein Academic Hospitals (2012 - 2016)

Since all the patients with congenital abnormalities are transferred from PTH to UAH there was no distinction made between these two hospitals. The above numbers can therefore be interpreted as the prevalence (2,1%) of significant congenital abnormalities, as well as the prevalence of the respective congenital abnormalities, in VLBW neonates in the Bloemfontein Academic hospitals between 1 January 2012 and 31 December 2016, as depicted by the below graph.

Of the 1585 VLBW neonates treated at PTH and UAH between 2012 and 2016, 34 (2,1%) had congenital abnormalities. The most common congenital abnormality seen in the excluded VLBW neonates in Bloemfontein Academic hospitals was duodenal Atresia (12%), followed by oesophageal atresia (12%).

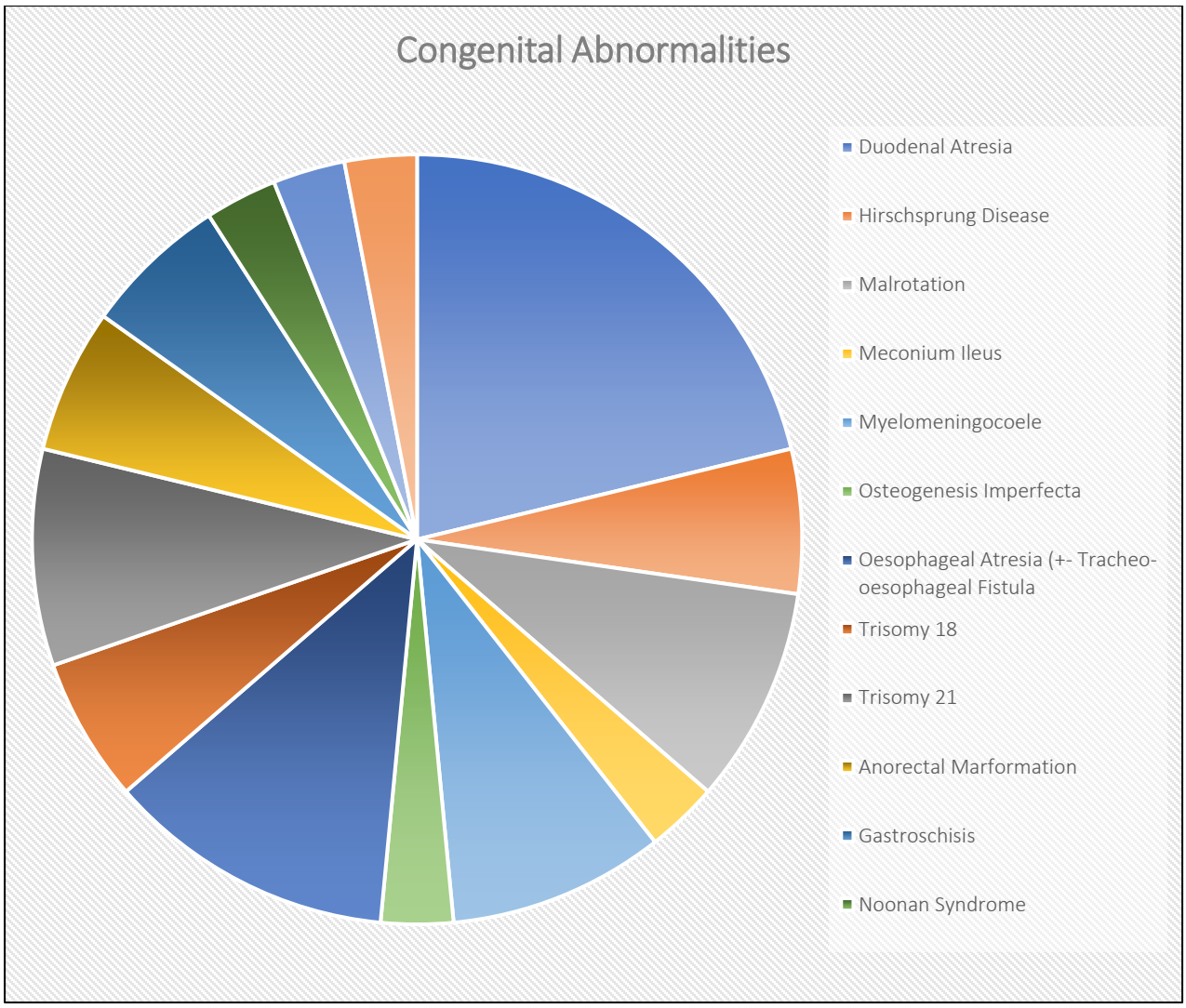

Figure 3: Distribution of congenital abnormalities in Very Low Birth Weight neonates excluded from the study. (2012 – 2016)

5.3 RBC transfusion results

5.3.1 Pelonomi Tertiary Hospital

The median birth weight of the 950 VLBW neonates whose data was evaluated at PTH, was 1300g, with a median gestational age of 30 weeks. Of these, 340 (36%) had RBC transfusions. The median birthweight for the latter was 1220g, with the median gestational age also 30 weeks. This indicates that the VLBW neonates who had received RBC transfusions were representative of the average VLBW neonate treated at PTH (p-value 0,01).

There was a marked change in RBC transfusion practices over time, with a decline in the number of VLBW neonates transfused at PTH between 2012 – 2016. In 2012, 114 (57%) of the 200 neonates received RBC transfusions, while in 2016 only 37 (19%) of the neonates were transfused (p-value 0,0005).

The median pre-transfusion haematocrit was 0.33 L/L, with the median post-transfusion haematocrit 0.40 L/L. The neonates who were NPO at the time of, and for at least 48-hours after the transfusion, was 172 (50,6%).

A marked change in the RBC transfusion practices can be seen after 2012 when only 40% of the neonates were kept NPO, compared to 2016 when a much larger percentage of 62% were kept NPO during and after transfusions. This can be correlated with the publication of new transfusion guidelines when TANEC became a more recognisable entity in the medical field.

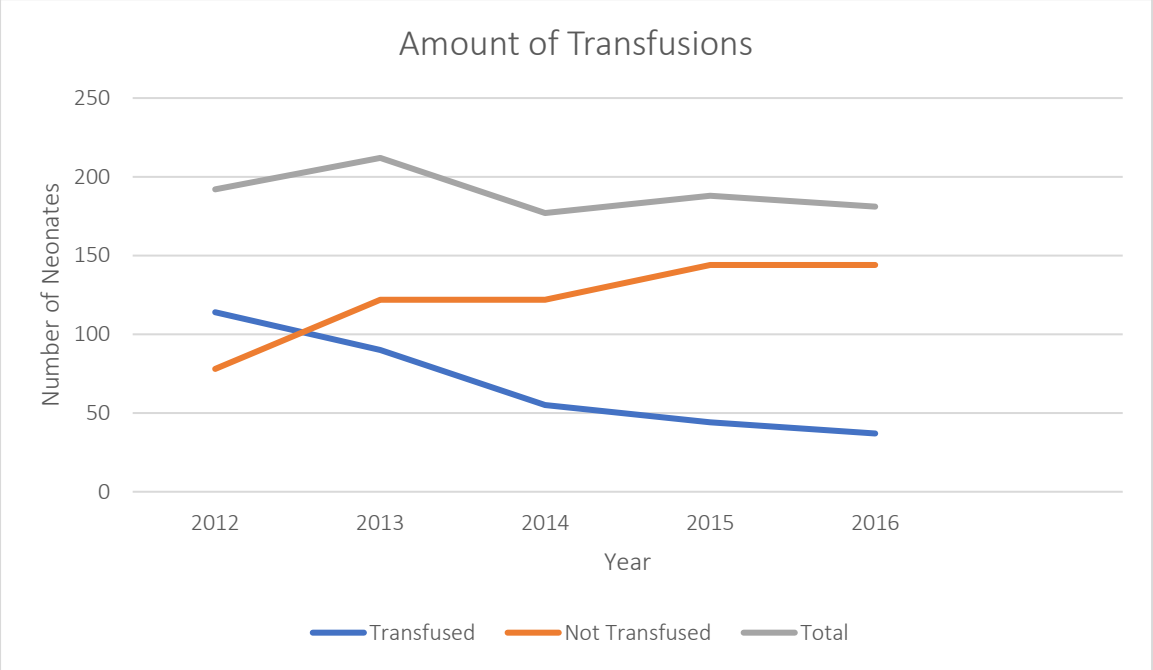

Figure 4: Amount of RBC transfusions in Very Low Birth Weight neonates at Pelonomi Tertiary Hospital (2012 - 2016)

The total number of VLBW neonates who received RBC transfusions at PTH slowly declined from 2012 to 2016, with a relative rise in the number of neonates kept NPO during transfusions. In 2012, 45 (40%) of the 114 neonates who received RBC transfusions were kept NPO, while in 2016, 23 (62%) of the 37 neonates who received transfusions were kept NPO.

The total number of VLBW neonates who received RBC transfusions at PTH continued to drop from 114 (57%) in 2012, to 37 (18,8%) in 2016. A percentage rise of 12% of neonates kept NPO during transfusions was demonstrated over the same period. This change in the number of patients kept NPO during RBC transfusion is statistically significant (p-value 0,03).

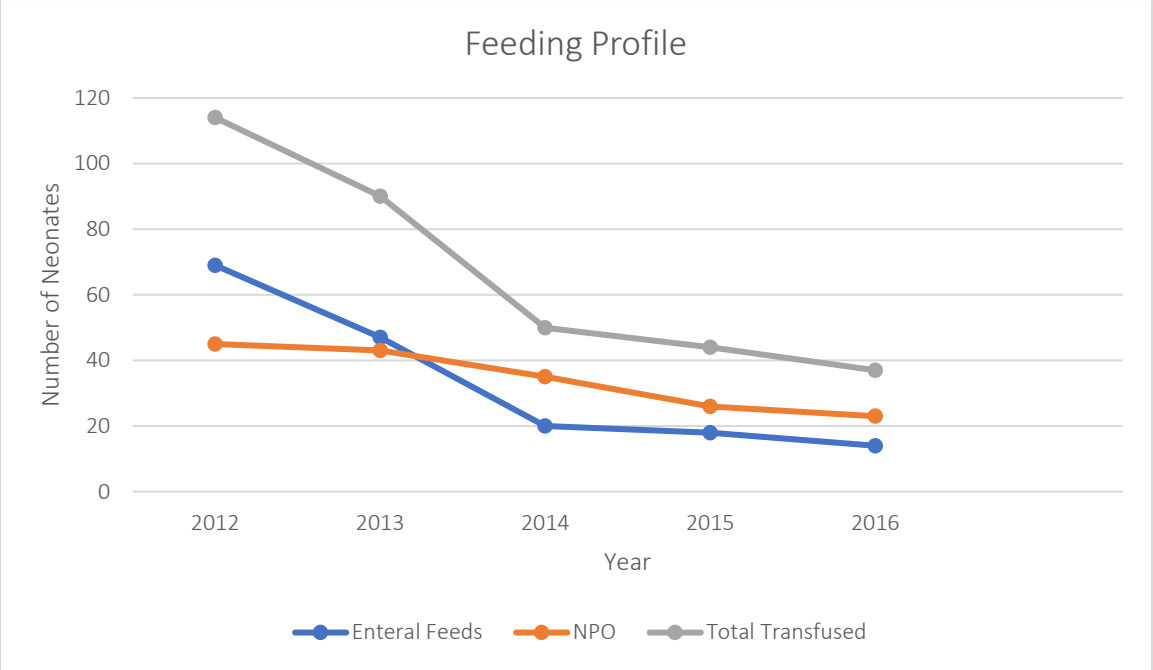

Figure 5: Feeding profile of Very Low Birth Weight neonates transfused at Pelonomi Tertiary Hospital (2012 - 2016)

The clinical conditions of neonates receiving RBC transfusions were the following:

Clinical condition	Number	Percentage
Stable (no clinical abnormality)	204	60%
Septic / Septic shock	40	11,8%
Respiratory distress	23	6,7%
Tachycardia	23	6,7%
Hypovolemic shock	23	6,7%
Apnoea	10	2,9%
Exchange transfusion	8	2,4%
Pulmonary haemorrhage	4	1,2%
Haemolysis	2	0,6%
Unstable (Specific clinical signs not specified)	2	0,6%
Tachypnoea	1	0,3%

Table 5: Clinical condition of Very Low Birth Weight neonates at Pelonomi Tertiary Hospital during RBC transfusion (2012 - 2016)

The vast majority (60%) of the transfused neonates was recorded as clinically stable and had no clinical indication for the RBC transfusion. When correlating this with the average pre-transfusion haematocrit (0,33L/L), neonates at PTH were transfused with RBCs more often than was clinically indicated.

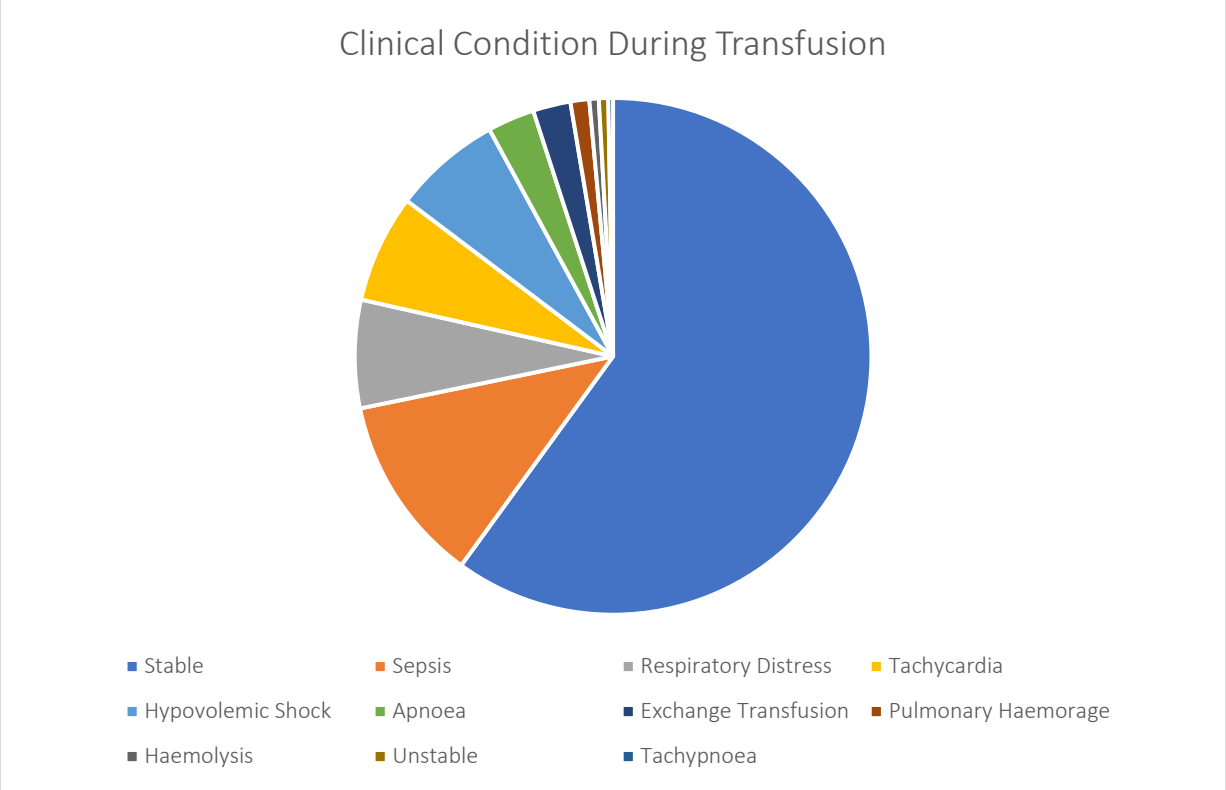

Figure 6: Distribution of clinical conditions of Very Low Birth Weight neonates at Pelonomi Tertiary Hospital during RBC transfusion (2012 – 2016)

The ventilatory support of the neonates who received RBC transfusions was also evaluated, and recorded as follows:

Ventilatory Support	Number	Percentage
High Frequency Oscillatory Ventilation	0 (Not available at PTH at time of study)	0%
Intermittent Positive Pressure Ventilation	30	8,9%
Nasal Continuous Positive Airway Pressure	70	20,6%
Nasal High Flow Oxygen	97	28,5%
Room Air	143	42%

Table 6: Ventilatory support in Very Low Birth Weight neonates at Pelonomi Tertiary Hospital during RBC transfusion (2012 - 2016)

The majority of transfused VLBW neonates (42%) were recorded to have had no respiratory problems and were stable on room-air. This is another pointer that RBC transfusion was not clinically indicated in most of the neonates.

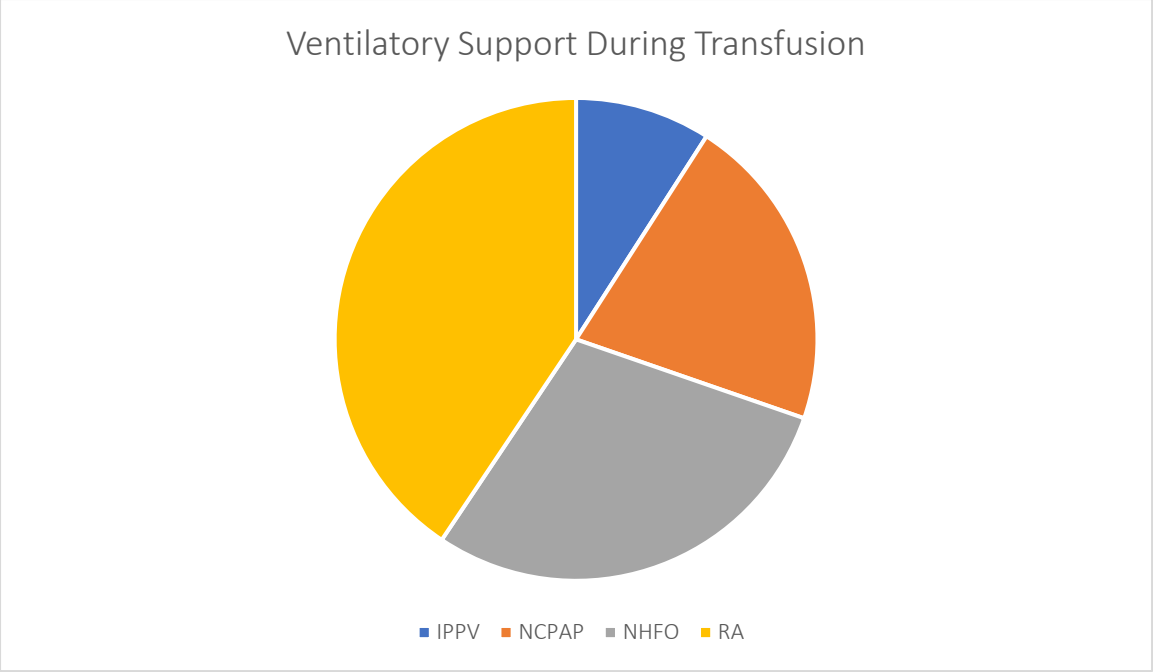

Figure 7: Ventilatory support in Very Low Birth Weight neonates at Pelonomi Tertiary Hospital during RBC transfusion (2012 – 2016)

5.3.2 Universitas Academic Hospital

The median birthweight of the included 476 VLBW neonates at UAH was 1200g, with a median gestational age of 29 weeks. Of these, 257 (54%) had RBC transfusions. The median birthweight for VLBW neonates who received RBD transfusions was 1160g, with the median gestational age being the same as for the group as a whole at 29 weeks. This indicates that the neonates who received RBC transfusions is representative of the average VLBW neonate treated at UAH (p-value 0,005).

The change in RBC transfusion practices at UAH was less dramatic than at PTH, although a steady decline in the number of transfused VLBW neonates was seen between 2012 and 2016. In 2012, 73 (62%) of the VLBW neonates received RBC transfusions, compared to only 32 (31%) in 2016. Since the number of VLBW neonates managed at UAH stayed relatively constant, between 104 and 117, the change in the number of RBC transfusions can only be explained by a change in transfusion practices in the unit (p-value 0,0003).

The median pre-transfusion haematocrit for these neonates was 0,33 L/L, whilst the median post-transfusion haematocrit was 0,41 L/L. The neonates who were kept NPO during, and for at least 120minutes after the RBC transfusion, was 110 (42,8%).

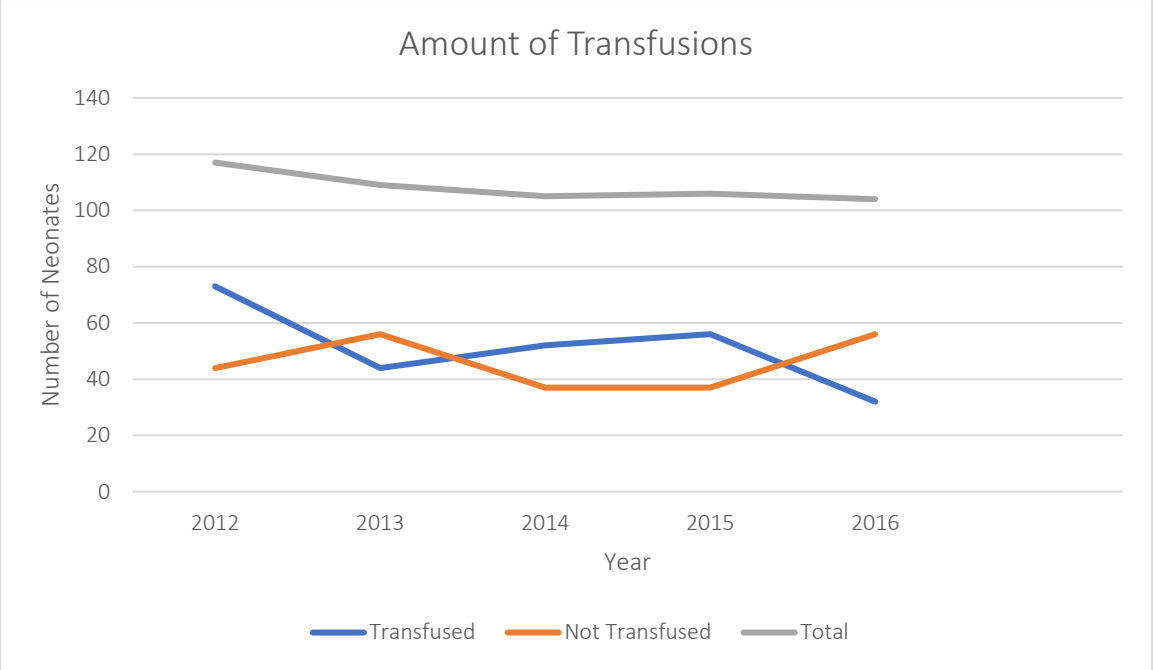

Figure 8: Amount of RBC transfusions in Very Low Birth Weight neonates at Universitas Academic Hospital (2012 - 2016)

The total number of VLBW neonates who received RBC transfusions at UAH dropped slightly from 2012 to 2016, with a percentage rise in the number of neonates who were kept NPO during their transfusions. In 2012, 21 (29%) of the 73 transfused neonates were kept NPO during the RBC transfusion, while in 14 (44%) of the 32 neonates who were transfused in 2016 were kept NPO during the transfusion. The change in the number of patients kept NPO during and after RBC transfusions over the 5-year period is statistically significant (p-value 0,008).

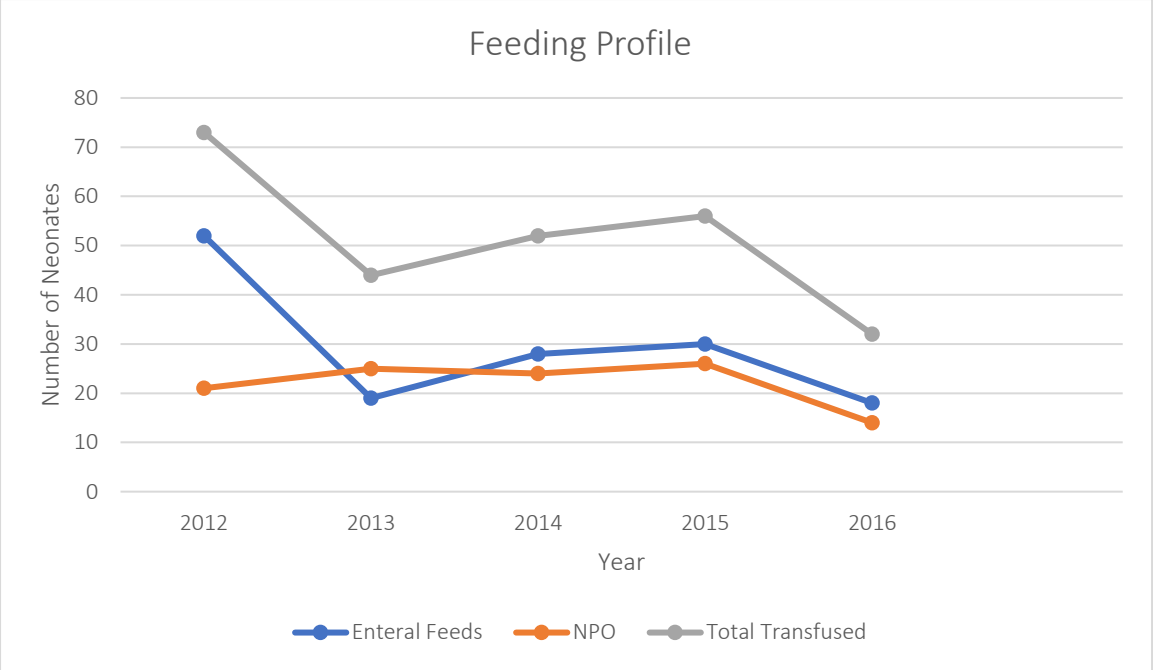

Figure 9: Feeding profile of Very Low Birth Weight neonates transfused at Universitas Academic Hospital (2012 - 2016)

The clinical conditions of neonates whilst receiving RBC transfusions were recorded as follows:

Clinical condition	Number	Percentage
Stable (No clinical abnormality)	155	60,3%
Septic / Septic shock	38	14,8%
Tachycardia	29	11,3%
Respiratory distress	15	5,8%
Hypovolemic shock	11	4,3%
Apnoea	4	1,6%
Tachypnoea	2	0,8%
Exchange transfusion	1	0,4%
Upper Gastro-intestinal Bleed	1	0,4%
Unstable (Specific clinical signs not specified)	1	0,4%

Table 7: Clinical condition of Very Low Birth Weight neonates at Universitas Academic Hospital during RBC transfusion (2012 - 2016)

The majority (60,3%) of the neonates who received RBC transfusions, was recorded to be clinically stable and therefore had no clinical indication for transfusion. This number is similar to the one for PTH. Likewise, the median pre-transfusion haematocrit was the same (0,33L/L), indicating that neonates at UAH received more RBC transfusions than clinically indicated, similar to those at PTH.

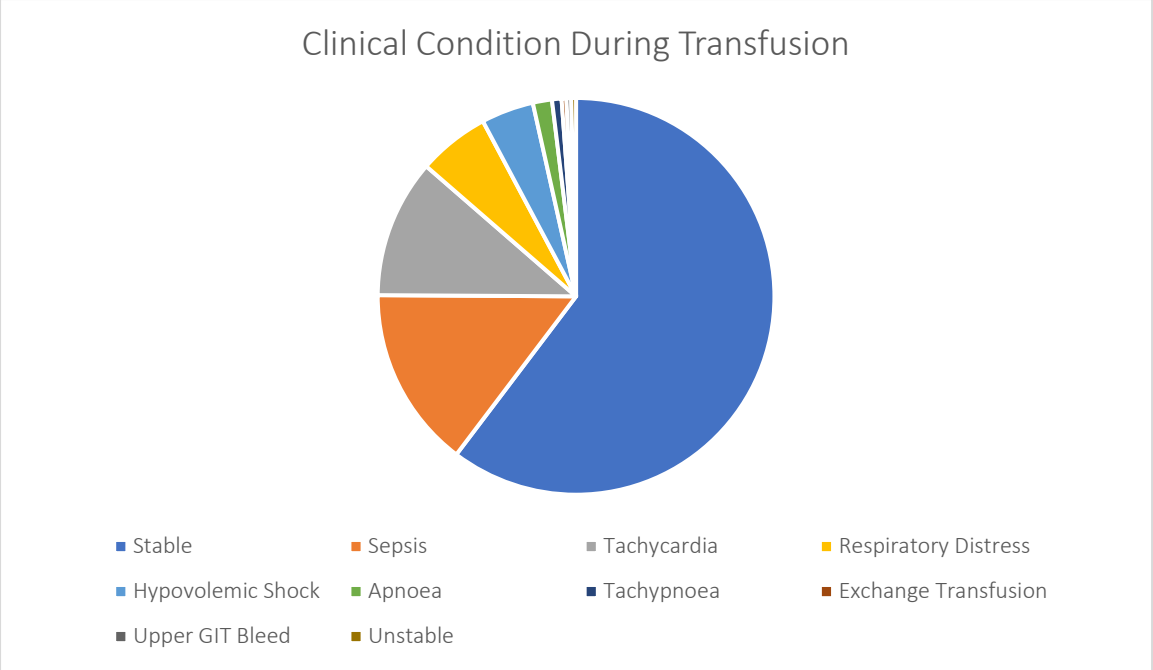

Figure 10: Distribution of clinical condition of Very Low Birth Weight neonates at Universitas Academic Hospital during RBC transfusion (2012 – 2016)

The ventilatory support of the neonates who received RBC transfusions was recorded as follows:

Ventilatory Support	Number	Percentage
High Frequency Oscillatory Ventilation	13	5%
Intermittent Positive Pressure Ventilation	18	7%
Nasal Continuous Positive Airway Pressure	28	11%
Nasal High Flow Oxygen	73	28,4%
Room Air	125	48,6%

Table 8: Ventilatory Support of Very Low Birth Weight neonates at Universitas Academic Hospital during RBC transfusions (2012 – 2016)

The majority of transfused VLBW neonates (48,6%) at UAH had no respiratory problems and were stable on room air. This again points to no clinical indication for RBC transfusion in most of the neonates.

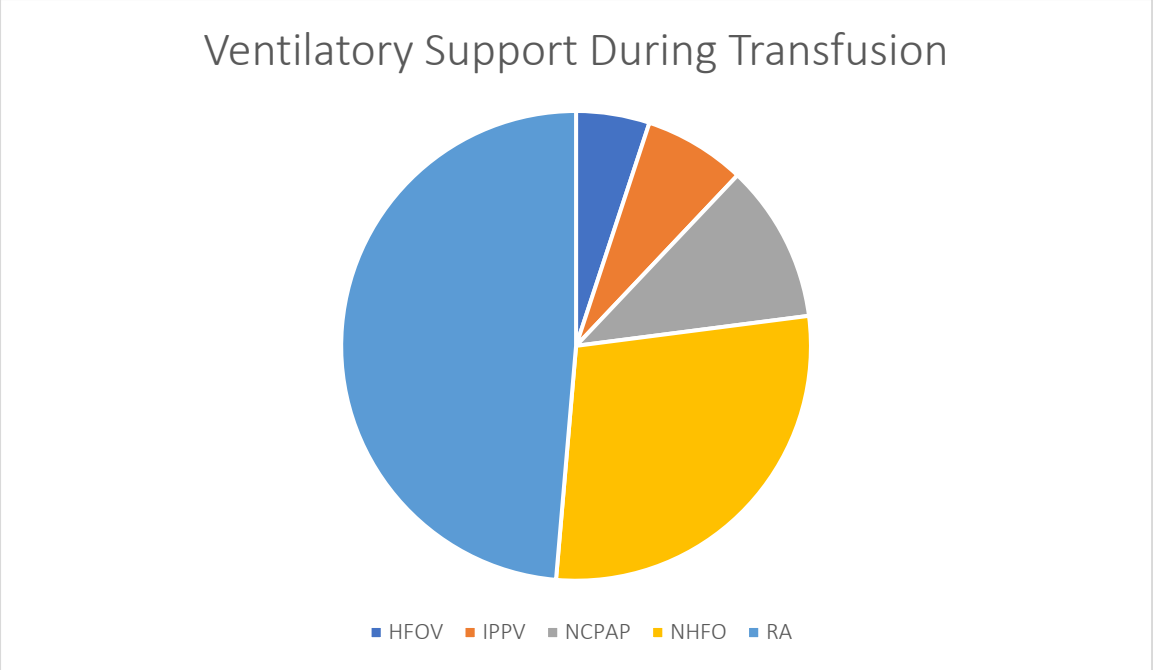

Figure 11: Ventilatory Support of Very Low Birth Weight neonates at Universitas Academic Hospital during RBC transfusions (2012 – 2016)

5.4 NEC results

5.4.1 Pelonomi Tertiary Hospital

Of the 950 VLBW neonates treated at PTH during the 5-year study period, 215 were recorded to have developed NEC. This accounts for an incidence of 22,6%, which is higher than the international standard incidence of 11% in VLBW neonates.[10] Of the 215, 119 (55,3%) developed NEC before receiving an RBC transfusion, while 96 (44,7%) developed TANEC. The majority (43%) of the 215 neonates who developed NEC were classified as Grade I according to the Modified Bell's Staging. The Bell's Staging breakdown was as follows.

Grade	Number	Percentage
I	92	43%
IIA	56	26,2%
IIB	30	14%
IIIA	7	3,3%
IIIB	29	13,5%

Table 9: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital (2012 - 2016)

To better understand any changes in the development of the NEC over the five recorded years, the years were looked at individually for severity of the NEC and any patterns that may have flagged throughout the years.

In 2012, 72 VLBW neonates developed NEC in PTH. The majority were classified as Grade I (34,7%), and 50 of these (69,4%) had developed TANEC. The total number of neonates who received RBC transfusions in 2012 was 114. Only 45 (39,5%) were kept NPO during and for at least 120minutes after the transfusion.

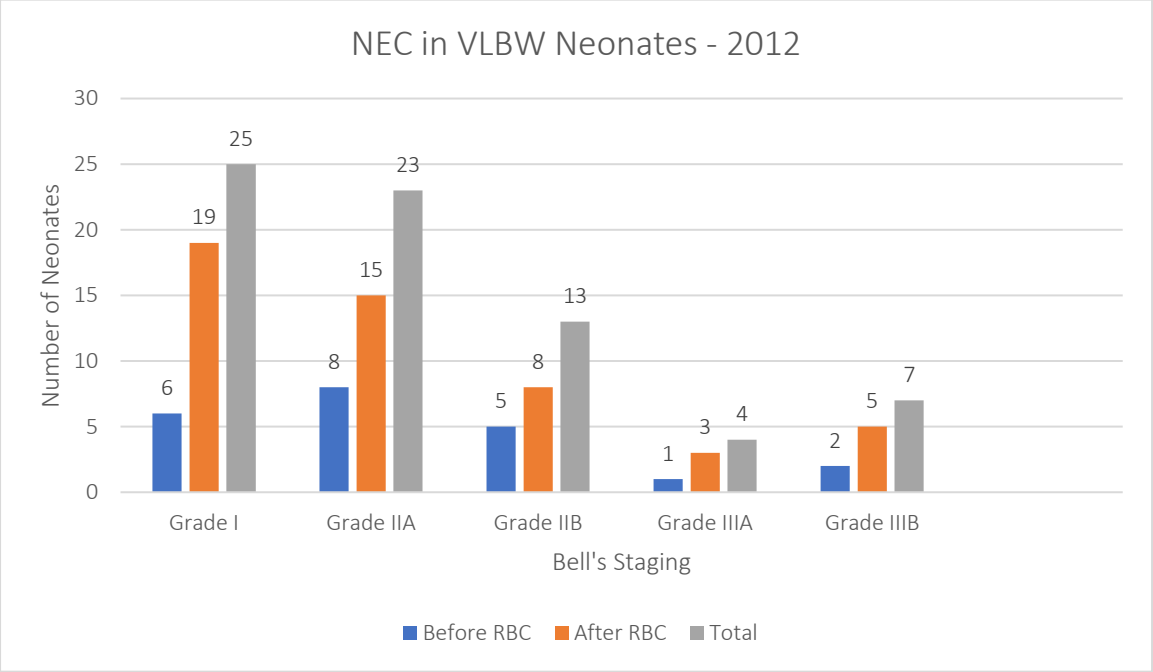

Figure 12: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital – 2012

A minor drop in NEC-cases was seen in 2013 with 52 in total at PTH. The majority of were still classified as Grade I (42,3%). The least frequent Bell's Staging grade was Grade IIIA (2%). There was an accompanying decrease in the percentage of neonates who developed TANEC (25 of the 52: 48%). The total number of neonates who received RBC transfusions dropped from 114 in 2012 to 90 in 2013, of which 43 (48%) were kept NPO during and for at least 120minutes after the transfusion.

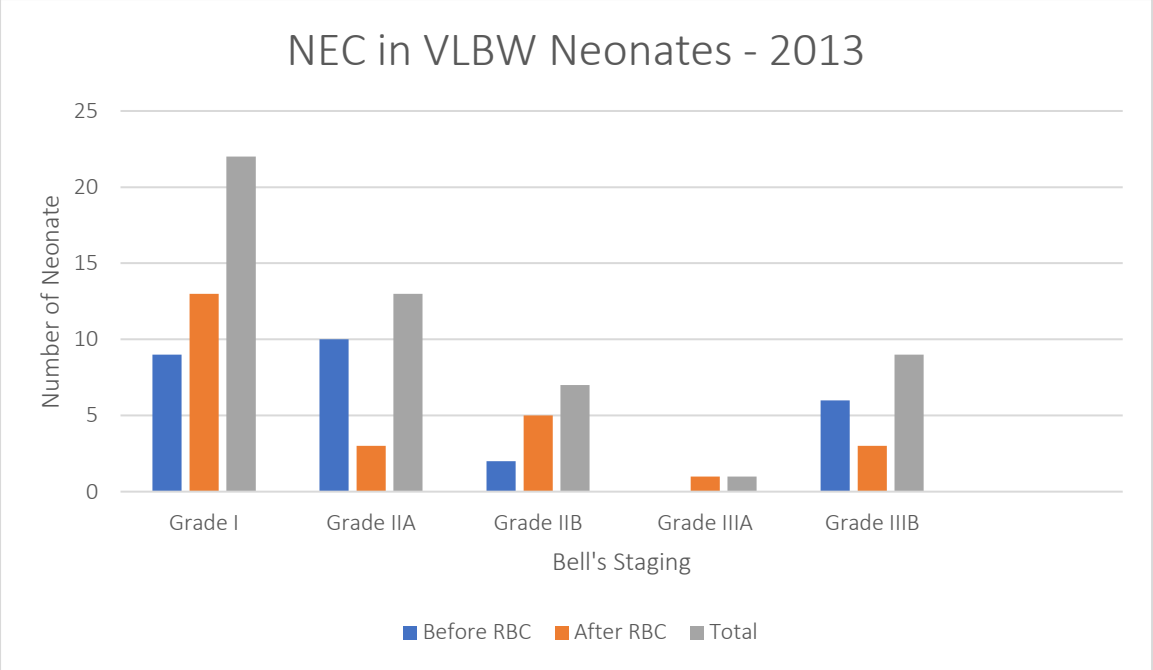

Figure 13: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital – 2013

In 2014 we saw another drop in the total number of VLBW neonates who developed NEC at PTH, when only 27 (13,4%) of the 201 neonates developed NEC. The majority were still classified as Grade I (52%), with the least frequent Bell's Staging grade continuing to be Grade IIIA. Of the 27 neonates who developed NEC, only 8 (29,6%) developed TANEC, a drop from the 25 (48%) in 2013. The total number of neonates who received RBC transfusions dropped from 90 in 2013 to 55 in 2014, of which 35 (63,6%) were kept NPO during and for at least 120minutes after the transfusion.

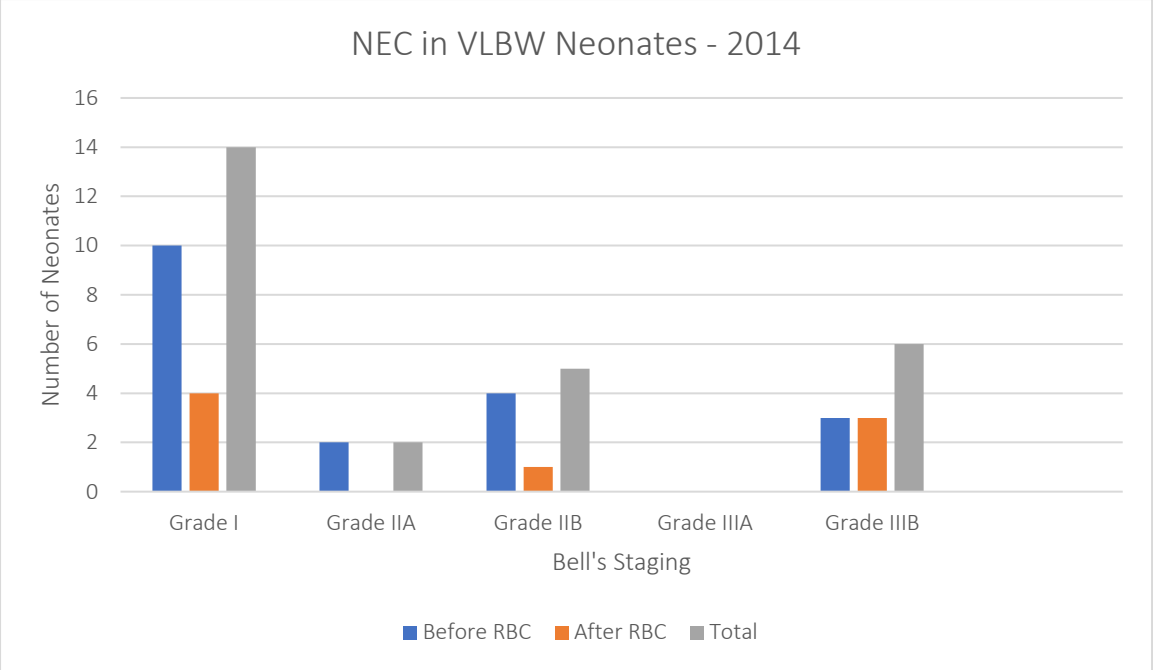

Figure 14: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital - 2014

In 2015, as in 2014, 27 of the 203 VLBW neonates developed NEC. Only 3 of these (11%) were TANEC, a further drop from the 8 in 2014. The total number of neonates who received RBC transfusions dropped from 55 in 2014 to 44 in 2015, continuing the persistent drop from 2012. Twenty-six (60%) were kept NPO during and for at least 120minutes after the transfusion.

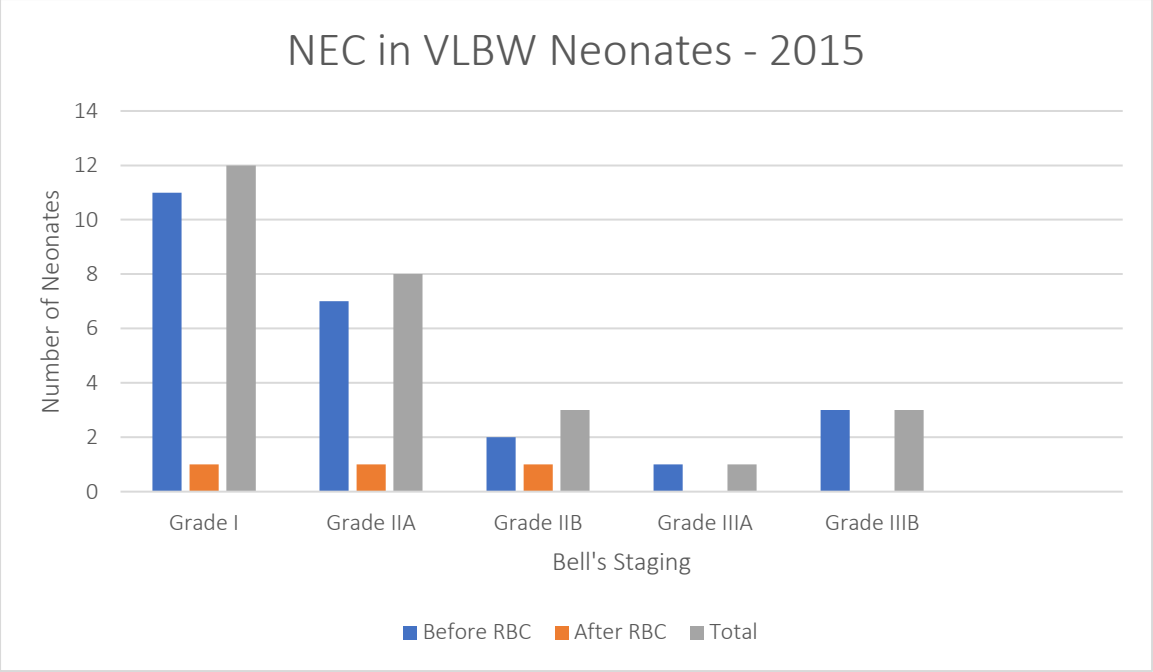

Figure 15: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital – 2015

2016 brought a slight increase in the number of VLBW neonates who developed NEC in PTH. Of the 36 neonates who developed NEC, only 7 (20%) had developed TANEC. The total number of neonates who received RBC transfusions dropped from 44 in 2015 to 37 in 2016. Of these neonates who received RBC transfusions, 23 (62%) were kept NPO during and for at least 120minutes after the blood transfusion.

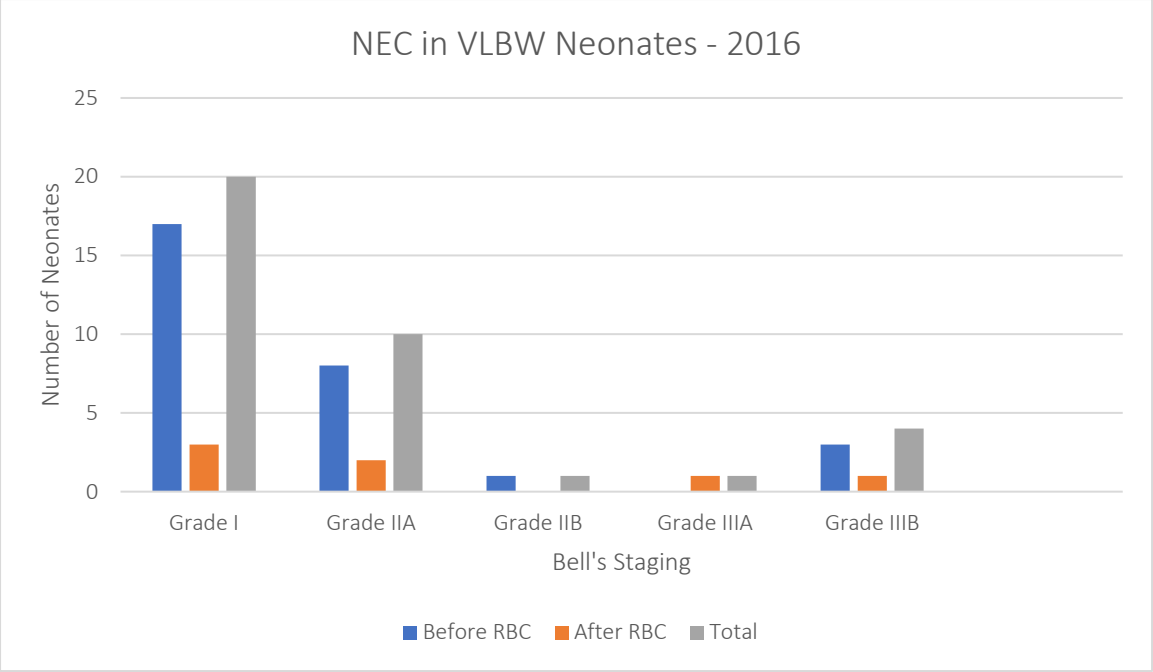

Figure 16: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital – 2016

Even though the incidence of NEC in VLBW neonates in PTH (22,6%) is higher than the international standard of 11% [10], a remarkable decrease was seen in the overall numbers over the 5-year study period. The majority of the neonates had developed NEC Grade I or II.

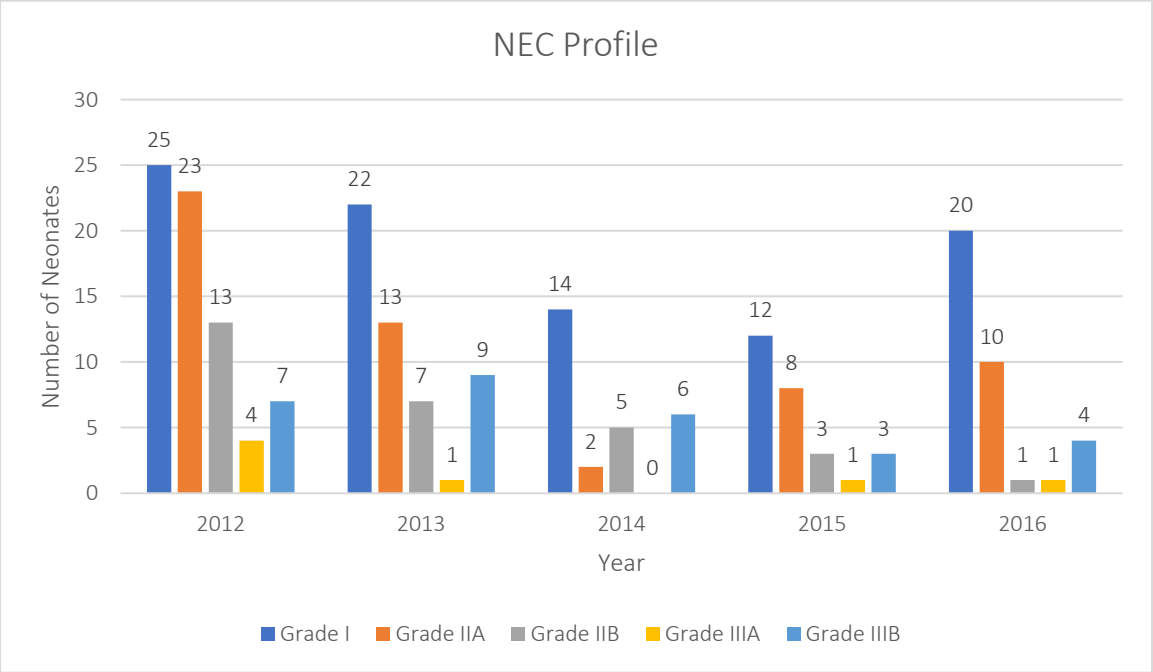

Figure 17: Modified Bell's Staging profile for Very Low Birth Weight neonates who developed NEC at Pelonomi Tertiary Hospital (2012 - 2016)

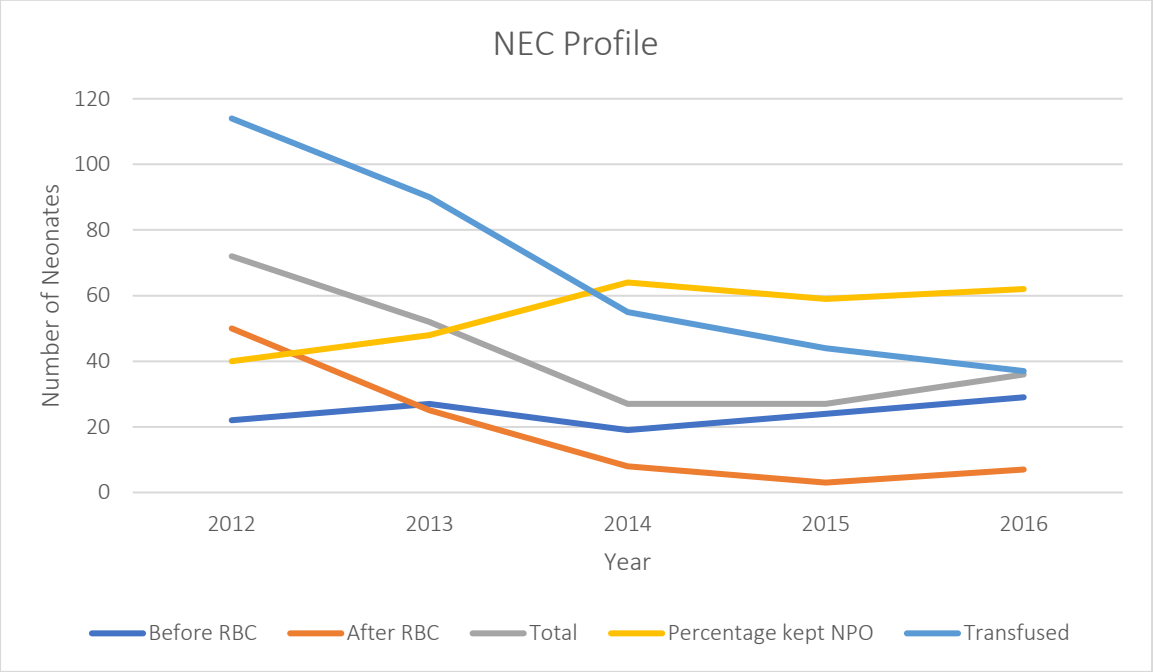

Figure 18: Profile of Very Low Birth Weight neonates who developed NEC at Pelonomi Tertiary Hospital (2012 - 2016)

Thus, a relative increase in the number of neonates kept NPO during RBC transfusion (40% in 2012 vs. 62% in 2016), coincided with a decrease in the total number of neonates transfused (114 in 2012 vs. 37 in 2016, p-value 0,03). This again coincided with a decrease in the number of neonates who developed TANEC (50 in 2012 vs. 7 in 2016, p-value 0.002). This correlation is of statistical significance (p-value 0,046).

5.4.2 Universitas Academic Hospital

Of the 476 VLBW neonates treated at UAH during the 5-year study period, 149 (31,3%) were recorded to have developed NEC. This accounts for 31,3% of the VLBW neonates, placing our incidence above the international standard of 11% [10]. Of these, 78 (52,3%) neonates developed NEC before receiving an RBC transfusion, and 71 (47,7%) developed TANEC. The majority (43,6%) was classified as Grade I according to the Modified Bell's Staging. The Bell's Staging breakdown was as follows:

Grade	Number	Percentage
I	65	43,6%
IIA	45	30,2%
IIB	22	14,8%
IIIA	2	1,3%
IIIB	15	10,1%

Table 10: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital (2012 - 2016)

To better understand any changes in the development of NEC over the five recorded years, the years were looked at individually for severity of the NEC, and any specific patterns that may have flagged throughout the years.

In 2012, a total of 38 VLBW neonates developed NEC at UAH. The majority were classified as Grade IIA. Thirty-one (81,6%) had developed TANEC. In 2012, the number of VLBW neonates who received RBC transfusions was 73, of which only 21 (28,8%) were kept NPO during and for at least 120minutes after the transfusion.

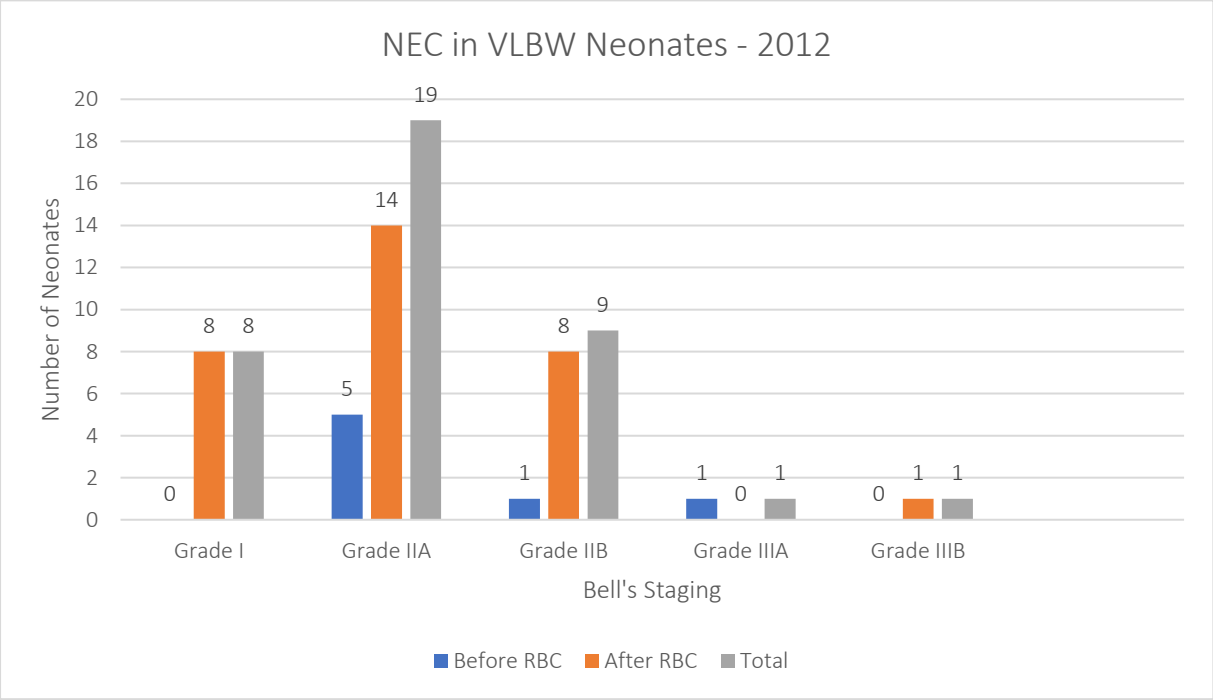

Figure 19: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2012

During 2013, 31 VLBW neonates developed NEC at UAH, comparing well with the 38 of 2012. The majority of these were classified as Grade I (45%), with none classified as Grade IIIA. Twelve of the 31 (38,7%) had developed TANEC, a major drop from the 31 (81,6%) in 2012. The total number of neonates who received RBC transfusions declined from 73 in 2012 to 44 in 2013, of which 25 (57%) were kept NPO during and for at least 120minutes after the transfusion. Only

28,8% of the neonates who received RBC transfusions in2012 were kept NPO during and for at least 120min after the transfusion.

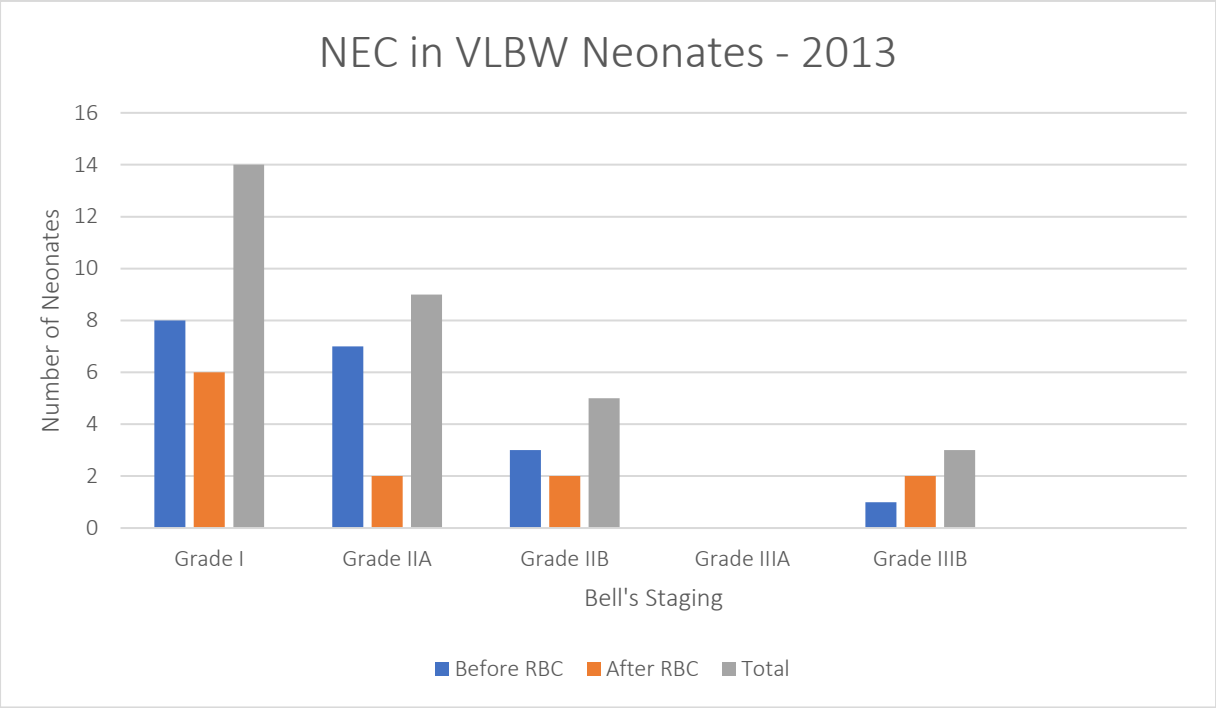

Figure 20: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2013

2014 saw another slight drop in the number of VLBW neonates who developed NEC at UAH, when only 26 of the 105 VLBW neonates developed NEC. The majority of these were still classified as Grade I (46%), with the least frequent Bell's Staging grade continuing to be Grade IIIA. Of the 26 neonates who had NEC, 12 (46%) had developed TANEC. The total number of neonates who received RBC transfusions increased slightly from 44 in 2013 to 52 in 2014. Of these 24 (46,2%) were kept NPO during and for at least 120minutes after the transfusion.

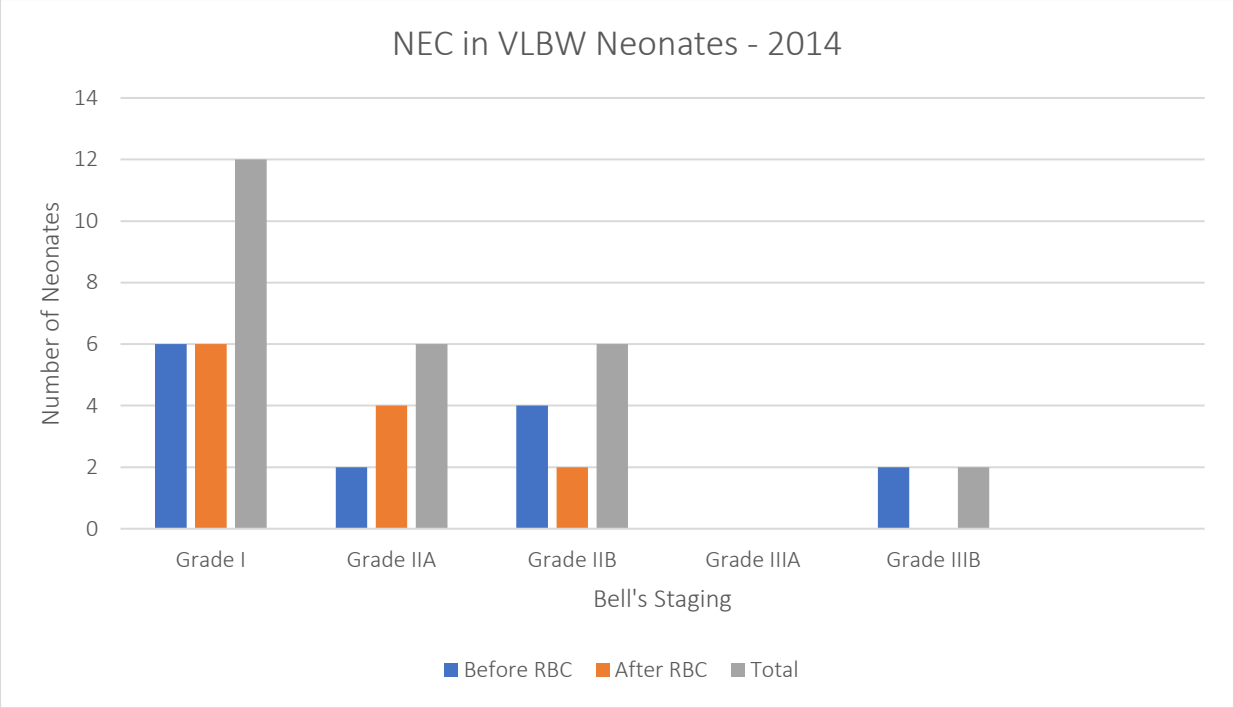

Figure 21: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2014

In 2015, 27 of the 93 VLBW neonates treated at UAH developed NEC. Of these, 10 (37%) had developed TANEC, largely unchanged from the 12 in 204. The total number of neonates who received RBC transfusions increased again from 52 in 2014 to 56 in 2015. Of these, 26 (46,4%) were kept NPO during and for at least 120minutes after the transfusion.

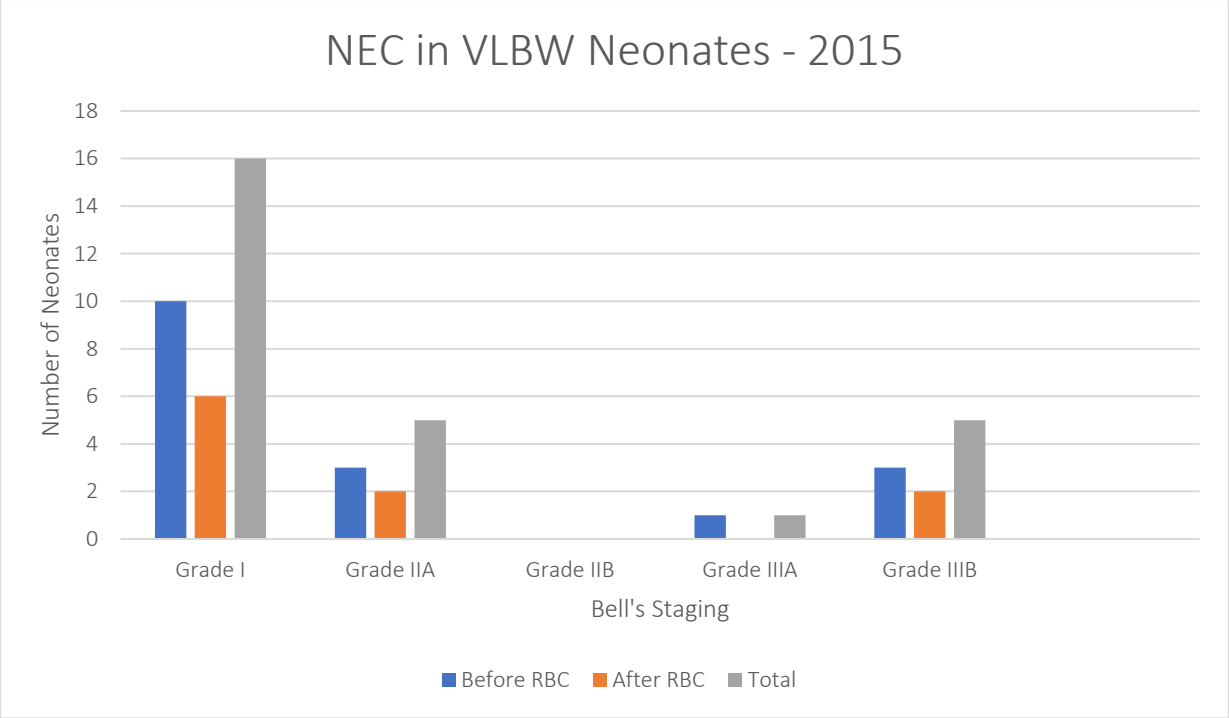

Figure 22: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2015

Lastly, in 2016 a persistent number of 27 VLBW neonates developed NEC at UAH. Of the 27 VLBW neonates who developed NEC, only 6 (22%) had developed TANEC. The total number of neonates who received RBC transfusions again dropped from 56 in 2015, to 32 in 2016. Of these VLBW neonates who received transfusions, 14 (44%) were kept NPO during and for at least 120minutes after the transfusion.

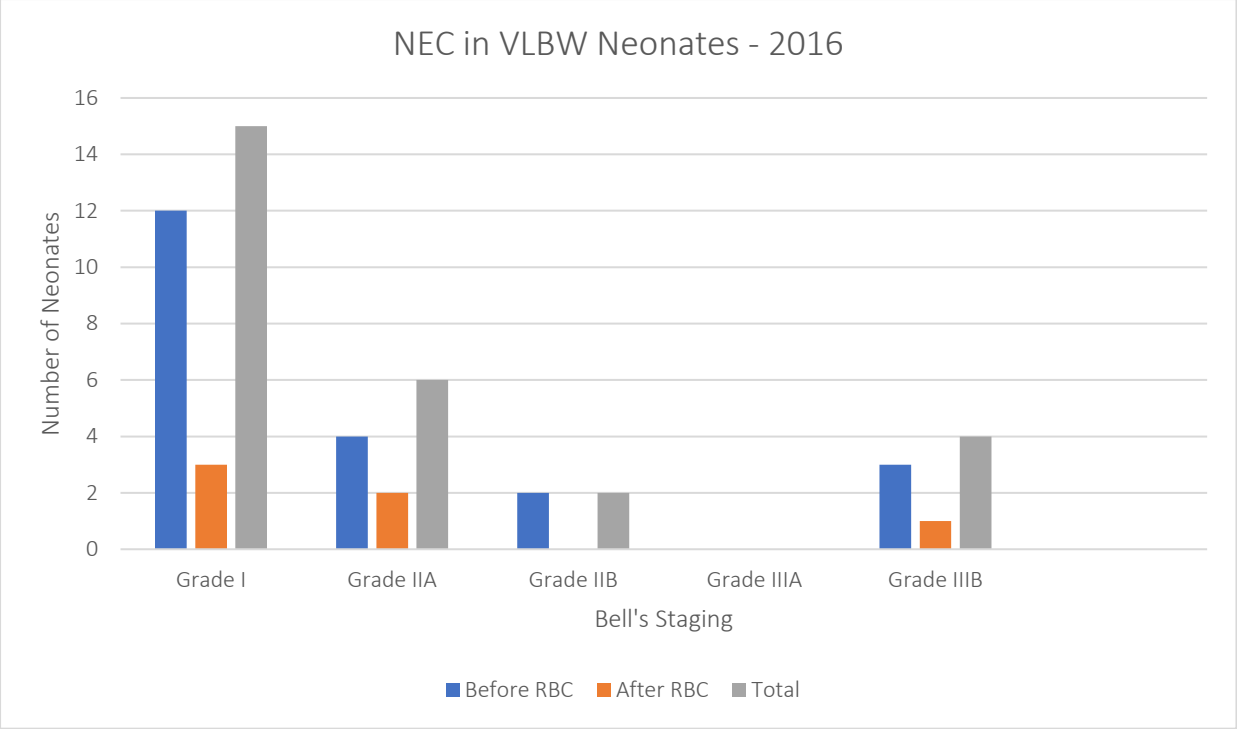

Figure 23: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2016

Thus, a relative increase in the number of neonates kept NPO during RBC transfusion (29% in 2012 vs. 44% in 2016), coincided with a decrease in the total number of neonates transfused (73 in 2012 vs. 32 in 2016, p-value 0,008). This also coincided with a decrease in the number of neonates who developed TANEC (31 in 2012 vs. 6 in 2016, p-value 0,0005). However, the correlation between neonates kept NPO during RBC transfusion, and those who developed TANEC at UAH, was not statistically significant (p-value 0,1673).

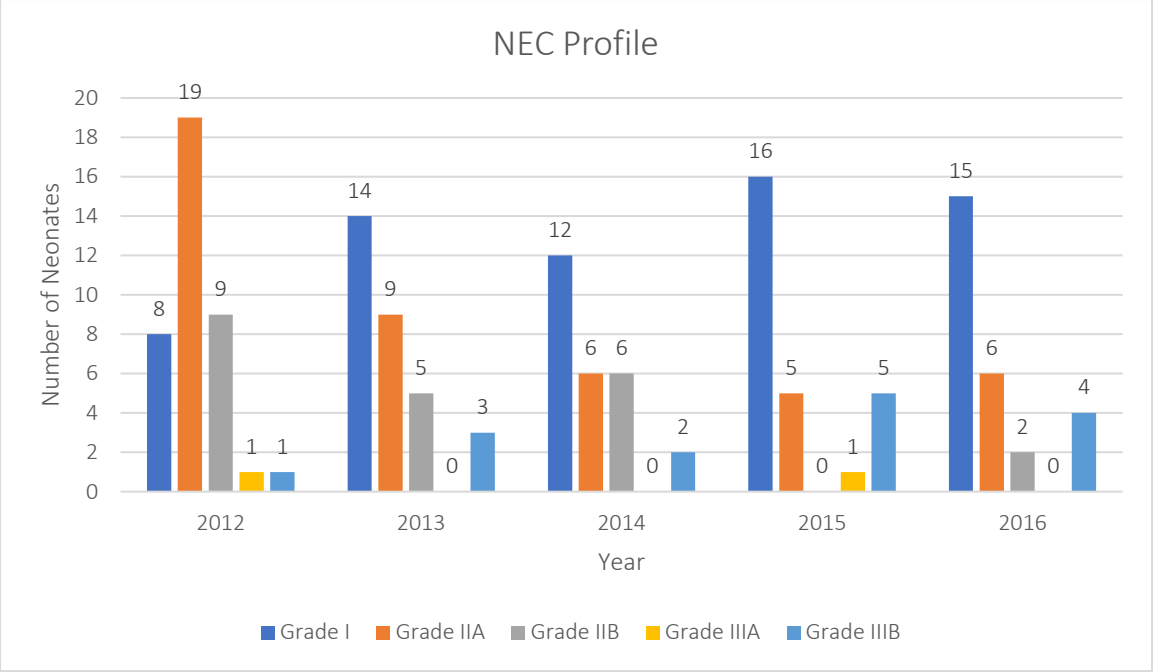

Figure 24: Modified Bell's Staging Profile for Very Low Birth Weight neonates who developed NEC at Universitas Academic Hospital (2012 - 2016)

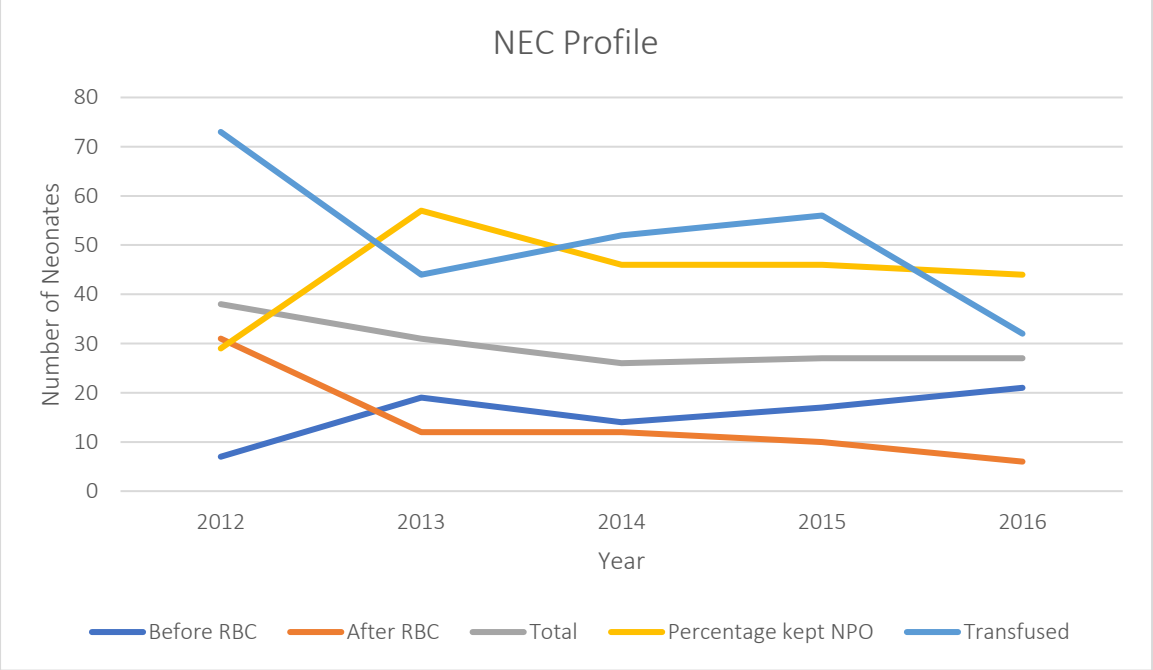

Figure 25: Profile of Very Low Birth Weight neonates who developed NEC at Universitas Academic Hospital (2012 - 2016)

Chapter 6: Discussion

Necrotizing enterocolitis (NEC) is one of the greatest disease burdens faced in neonatal units, and the most common acquired gastrointestinal pathology encountered in Very Low Birth Weight (VLBW) neonates. Despite the high incidence and subsequent morbidity and mortality associated with this disease, there is still a great paucity in our knowledge regarding its aetiology and pathology.

This lack of knowledge is not due to a lack of research in the field, as can be seen by the multitude of research efforts that has been made in the last 40 years. Research is especially critical in units with a high incidence of NEC, where the current disease burden should be utilized to further our knowledge of the pathophysiology and enable better management and hopefully prevention of this devastating disease.

6.1 Epidemiological data

The number of VLBW neonates treated at both Pelonomi Tertiary Hospital (PTH) and Universitas Academic Hospital (UAH), stayed relatively constant from 1 January 2012 to 31 December 2016. Thus, the number of VLBW neonates treated in both these academic hospitals served as a large enough study sample for the purposes of this study.

The prevalence of congenital abnormalities was determined before applying the exclusion criteria for the study. The most common surgical congenital abnormality in VLBW neonates was duodenal atresia (21%,) while oesophageal atresia (with or without tracheo-oesophageal fistulae) was the second most common with an incidence of 12%. The most common non-surgical congenital conditions were Trisomy 21 (9%), and Trisomy 18 (6%). There are very few recent studies looking specifically at the incidence of congenital surgical abnormalities in VLBW neonates specifically, so we were not able to correlate our findings with any other significant literature. The number of neonates excluded from the study did not significantly decrease the number of neonates that were included in the study.

6.2 RBC transfusions

Given the fact that the numbers in our VLBW neonatal population did not change much over the 5-year period, any changes in RBC transfusion numbers can directly be linked to changes in transfusion practices in the units. However despite these changes in transfusion practices, the majority of VLBW neonates who received RBC transfusions in these two academic hospitals were transfused at higher haematocrits (median 33%) than the current international guidelines,[61] as well as during stable clinical conditions (Table 2). It is therefore mandatory that a closer look be taken at the local RBC transfusion practices. The compilation of a scientific protocol to decrease the amount of unnecessary RBC transfusions, should be priority.

Indications for Transfusion of RBC's in Neonates	
HCT	AGE and CLINICAL CONDITION
<35%	≤1week of Life **AND** Unstable*
<28%	≤1week of Life **OR** Unstable*
<20%	>1week of Life **AND** Unstable*
*Unstable clinical condition is defined as a state of poor oxygen delivery, e.g. Respiratory distress/apnoea, Cardiovascular deterioration etc.	

Table 11 : Indications for RBC Transfusions in neonates [61]

Two separate studies pointed towards a relationship between RBC transfusions at lower haematocrit levels, and the development of TANEC [7763]. A study similar to this one done in 2015 showed a significantly lower pre-transfusion haematocrit for their patients who had developed TANEC (0,24L/L).[63] In a study on the effects of RBC transfusions in VLBW neonates done by Valieva et al in 2009, their patients had a mean pre-transfusion haematocrit of only 0,28L/L.[61] The median pre-transfusion haematocrit of 0,33L/L at the units in our study, is therefore much higher than those in the current literature.

There was a steady decrease in the number of VLBW neonates who received RBC transfusions in both our local hospitals over the five years study period, pointing towards a definite change in transfusion practices. The total number of VLBW neonates who received RBC transfusions at PTH persistently dropped from 114 (57%) in 2012, to 37 (18,8%) in 2016. During the same time at UAH the numbers declined from 73 (62%) in 2012, to 32 (31%) in 2016. Despite the high local RBC transfusion rates, it still undercuts most of the large international studies done on transfusions in neonates, where transfusion rates between 66% and 90% have been recorded.[61,78]

An increasing percentage of the VLBW neonates receiving RBC transfusions are kept NPO during, and for at least 120minutes after the transfusion, in response to the latest publications on TANEC. In 2012 only 39,5% of the VLBW neonates who received RBC transfusions at PTH were kept NPO during the transfusion. This figure steadily rose over the 5-year period, reaching a high of 62% in 2016.

A similar picture could be seen at UAH where only 28,8% of VLBW neonates who received RBC transfusions were kept NPO in 2012, while 44% were kept NPO in 2016. However, this is still not adequate compared to other studies where up to 83% of neonates who received RBC transfusion were kept NPO.[59,78]

6.3 Necrotising Enterocolitis

Even though the incidence of NEC in the two academic units in Bloemfontein (25,5%) is higher than the international standard of 11%, there has been a great decrease in the overall incidence of NEC over the 5-year study period, with the vast majority of the neonates developing Grade I (43,5%) or Grade II (41,3%) NEC.

A study conducted by Singh et al. in 2011 found the incidence of NEC in VLBW neonates in their institution to have been 3,67%. This is much lower than our incidence of 22,6% in PTH, and 31,3% at UAH, with a combined overall incidence of 25,5%.[63]

There is a statistically significant correlation between the change in numbers of neonates who received RBC transfusions, and the number of neonates who developed TANEC in PTH (p-value 0,0005) and UAH (p-value 0,0005) respectively, as well as for the combined VLBW population (p-value 0,0005).

A statistically significant correlation was found between the number of VLBW neonates who developed TANEC, and those who were kept NPO during and after their RBC transfusions at PTH (p-value 0,046), but not for the VLBW neonates at UAH (p-value 0,1673). This leads to the overall VLBW population not having a significant correlation between the change in those who were kept NPO, and those who developed TANEC (p-value 0,078).

6.3.1 Pelonomi Tertiary Hospital

In 2012 the practice of keeping patients NPO during RBC transfusions had not been thoroughly established yet. This could account for the fact that more than half of the neonates who developed NEC had done so after an RBC transfusion (69,4%). In 2013 the number of neonates not kept NPO during RBC transfusions was still quite high (48%).

In 2014 an increased number of neonates where kept NPO during RBC transfusions (63,7%). At the same time, a decrease in the number of neonates who developed TANEC was noted (29,6%).

Similarly, in 2015 a drop in the total number of neonates who received RBC transfusions was recorded, with only 11,1% who developed NEC within 48-hours after receiving an RBC transfusion. Another increase in the number of patients kept NPO during their RBC transfusion (60%) coincided with this decrease in TANEC, which supports the hypothesis that keeping VLBW neonates NPO during RBC transfusions will lead to a lower incidence of TANEC.

Thus, a relative increase in the number of neonates kept NPO during RBC transfusion (40% in 2012 vs. 62% in 2016), coincided with a decrease in the total number of neonates transfused (114 in 2012 vs. 37 in 2016, p-value 0,03). This also coincided with a decrease in the number of neonates who developed TANEC (50 in 2012 vs. 7 in 2016, p-value 0,002). This correlation is of statistical significance (p-value 0,046).

6.3.2 Universitas Academic Hospital

Between 2012 and 2013 a change in the RBC transfusion practices at UAH could already be seen as the number of neonates who received RBC transfusions dropped from 73 (62,4%) in 2012, to 44 (40,3%) in 2013. More neonates were also kept NPO during their RBC transfusions, with only 28,8% kept NPO in 2012 versus 57% kept NPO in 2013. This change in transfusion practices coincided with a drop in the number of neonates who developed TANEC, namely 81,6% in 2012 versus 38,7% in 2013.

2014 brough a slight increase in the number of RBC transfusions and a decreased percentage of them were kept NPO during the transfusions. With 49,5% of the VLBW neonates who received RBC transfusions, and only 46,2% kept NPO. This coincided with a percentage increase in the

number of neonates who developed TANEC, supporting the theory that feeds during RBC transfusions increases the risk for VLBW neonates developing TANEC.

Finally, a drop in the number of VLBW neonates who received RBC transfusions was seen in 2016, with the lowest percentage recorded at UAH over the study period of 30,8%. However, the percentage of these neonates who were kept NPO during their RBC transfusion dropped to 44%, and the number of VLBW neonates who developed TANEC also dropped to 22%.

A relative increase in the number of neonates kept NPO during RBC transfusion (29% in 2012 vs. 44% in 2016), coincided with a decrease in the total number of neonates transfused (73 in 2012 vs. 32 in 2016, p-value 0,008), which also coincided with a decrease in the number of neonates who developed TANEC (31 in 2012 vs. 6 in 2016, p-value 0,0005). However, the correlation between neonates who were kept NPO during transfusion, and those who developed TANEC, was not statistically significant at UAH (p-value 0,1673).

6.4 Limitations of this Study

Potential limitations of this study include its retrospective uncontrolled design. Some information might have been missed by the treating physicians at the time of hospital stay, and not included in the online Meditech© notes, and would thus not have been accessed.

Chapter 7: Conclusion and Suggestions

Necrotising enterocolitis is by far the largest surgical and gastro-intestinal burden faced by neonatologists and paediatric surgeons in Academic Hospitals in Bloemfontein. The local incidence is much higher than the global accepted average, giving rise to a greater responsibility to find ways in which to improve the current management. It is the leading cause of surgical deaths in our local VLBW population, by far outnumbering the amount of other congenital surgical abnormalities seen in the neonatal population. NEC is also a disease that causes a multitude of long-term complications, including short bowel syndrome, and poor neurological outcomes.[27,76]

As with most medical conditions, initially the focus of care was on managing the NEC once it has already developed. Medical knowledge has however moved on to a more advanced era, where doctors caring for neonates, are no longer merely interested in managing the problems once they arise. On the contrary, they are increasingly interested in preventing the conditions from arising in the first place.

Since the exact aetiology of NEC is still unknown, further studies are necessary to find possible causes, as well as contributing factors.

The authors are of the opinion that a strict protocol for RBC transfusion in neonates should be established at all hospitals. The changes in RBC transfusion practices witnessed over the 5-year period in the two academic hospitals in Bloemfontein, Free State, proved a definite correlation between RBC transfusions in VLBW neonates, and the development of TANEC (NEC within 48hours after the transfusion). Even though the steady decrease in numbers of VLBW neonates transfused over the 5-year period, already indicates a move in the right direction, an unacceptable number of neonates are still transfused without any clinical indication, causing an unnecessary risk for development of NEC and other RBC transfusion related morbidities.

Looking at the results of this data collection, one can postulate that an improvement in RBC transfusion practices, by means of a protocol, will lead to not only preventing unnecessary transfusions, thus saving blood products, a limited resource in this country, and money but also protecting at risk neonates from developing a potentially fatal disease.

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A. Ethics Approval Letter

B. FSDoH Approval Letter

C. HoD Approval Letter

D. Research Protocol

E. Data form

F. Turnitin digital receipt

G. Turnitin similarity report

Appendix A

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

09-Apr-2018

Dear **Dr Iselma Kruger**

Ethics Clearance: **Transfusion Practices in Very Low Birth Weight Neonates, and the Development of Necrotizing Enterocolitis, in two Neonatal Units in Bloemfontein, Free State.**

Principal Investigator: **Dr Iselma Kruger**

Department: **Surgery (Bloemfontein Campus)**

CONDITIONALLY APPROVED

With reference to your application for ethical clearance with the Faculty of Health Sciences, this letter is to inform you on behalf of the Health Sciences Research Ethics Committee that ethical clearance will be granted for your research, pending clarifications/submission of the following:

Permission from Department of Health outstanding. Please submit

Please note: You have 30 calendar days from date of issuance to respond to this letter. If no response has been received by HSREC Administration within this time, this application will be withdrawn from further consideration and you will have to reapply.

Your ethical clearance number will be issued as soon as the HSREC has reviewed and approved your response to the above mentioned stipulations. For the time being, please use the following RIMS reference number in all correspondence:**UFS-HSD2017/0616**

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance. We look forward to receiving your response.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 00006240; REC 230408-011; IORG0005187; FWA00012784
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
www.ufs.ac.za

Appendix B

health
Department of Health
FREE STATE PROVINCE

14 August 2018

Dr I Kruger
Dept. of Surgery
UFS

Dear Dr I Kruger

Subject: Transfusion Practices in Very Low Birth Weight Neonates, and the Development of Necrotizing Enterocolitis, in two Neonatal Units in Bloemfontein, Free State.

- Please ensure that you read the whole document, Permission is hereby granted for the above – mentioned research on the following conditions:
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of Pelonomi Hospital nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- **Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).**
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** lithekom@fshealth.gov.za or sebeelats@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- Please discuss your study with the institution manager/CEOs on commencement for logistical arrangements
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- You are encouraged to present your study findings/results at the Free State Provincial health research day
- Future research will only be granted permission if correct procedures are followed see http://nhrd.hst.org.za

Trust you find the above in order.
Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 30/8/18

Head : Health
PO Box 227, Bloemfotein, 9300
4th Floor, Executive Suite, Bophelo House, cnr Maitland and, Harvey Road, Bloemfotein
Tel: (051) 408 1646 Fax: (051) 408 1556 e-mail:khusemj@fshealth.gov.za@fshealth.gov.za/chikobvup@fshealth.gov.za

Appendix C

UNIVERSITY OF THE
FREE STATE
UNIVERSITEIT VAN DIE
VRYSTAAT
YUNIVESITHI YA
FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

08 May 2017

The Health Research Ethics Committee
University of the Free State
Bloemfontein

Dear Me Marais

Re: Permission for a research project

I herewith acknowledge Dr I Kruger's proposed research project under the supervision of Dr E Brits on:

Transfusion associated necrotising enterocolitis: A retrospective review of practices in two neonatal intensive care units in Bloemfontein

and grant permission to do this research in the Paediatric Surgery Unit.

Yours sincerely

SM LE GRANGE
(Head: Paediatric Surgery)

Head of Department/Departementshoof: Prof RS du Toit
Prof in Surgery/Prof in Chirurgie: Prof R Barry
Consultants/Konsultante: Prof SJA Smit, CA Loubser, SM le Grange, E Arko-Cobbah, DP Menge, NE Pearce, VC Simmons, PJ Oosthuizen, W Joubert

Department of Surgery/ Departement Chirurgie
Medical School/Mediese Skool
T: +27(0)51 405 3545, F: +27(0)51 444 1023, E: dutoitrs@ufs.ac.za
205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
P.O. Box/Posbus 339, Bloemfontein 9300, South Africa/Suid-Afrika, www.ufs.ac.za

Appendix D

Research Study Protocol

Transfusion Practices in Very Low Birth Weight Neonates, and the Development of Necrotising Enterocolitis, in two Neonatal Units in Bloemfontein, Free State.

Research Study Protocol

Transfusion Practices in Very Low Birth Weight Neonates, and the Development of Necrotising Enterocolitis, in two Neonatal Units in Bloemfontein, Free State.

Investigator:

Dr Iselma Kruger

MP0744522

Student Number: 2006013698

Registrar: Paediatric Surgery

Faculty of Health Science

School of Medicine

University of the Free State

Bloemfontein

Tel: 072 693 1076

Study Leader:

Dr E. Brits

Consultant Paediatric Surgeon

Department of Paediatric Surgery

Faculty of Healthy Science

School of Medicine

University of the Free State

Bloemfontein

Tel: 083 413 0068

Index

1. Introduction

Necrotising enterocolitis (NEC) places a massive burden on neonatal units and the healthcare system. Not only does it significantly prolong the hospital stay of neonates, it also causes detrimental long term sequelae, such as neurodevelopmental delay, growth retardation and chronic gastrointestinal complications(1). However, despite it being a well-documented disease, with the first reports of clinical findings indicative of NEC being made as early as the early 1800's (2), it continues to elude neonatologists in terms of risk factors, prevention and treatment strategies.

Further research is necessary to help identify possible contributing factors, so as to improve treatment, as well as to develop preventative strategies for this difficult condition. One possible contributing factor that has been identified by researchers, is the transfusion of blood products in the neonatal period, especially in preterm neonates. Since there is currently no standardised transfusion protocols in the two Neonatal Intensive Care Units (NICU's) in Pelonomi Hospital, and Universitas Academic Hospital, patients receive transfusions at a great variety of different clinical stages. By retrospectively evaluating the transfusion practices in these units, in particular in those patients who developed NEC, we hope to gain better insight into the possible causative relationship between blood transfusions, and the development of NEC.

2. Literature Study

2.1 Definition of Terms:

Neonate:
Any new-born baby up to the age of 28 days (1).

Preterm Neonate:
Any neonate born to a mother who has been pregnant for less than 37 week (1).

Necrotising Enterocolitis (NEC):
Necrotising enterocolitis is cellular death (necrosis) of the bowel of a neonate, diagnosed by utilizing clinical and radiographic findings, as described in a later section (Diagnosis).

Transfusion Associated Necrotising Enterocolitis (TANEC):
Transfusion Associated Necrotising Enterocolitis; NEC that occurs in a patient within 48hours after receiving a transfusion of packed red blood cells (3).

Very Low Birth Weight Infants (VLBW):
Any neonate born at a weight of between 1000g and 1499g (4).

Enteral Feeds:
Nutritional substances (feeds) delivered to the patient directly via the gastro-intestinal tract (thus either via the mouth, or directly into the stomach or small intestine) (4).

Packed Red Blood Cells (PRBC's):
Packed red blood cells. Donor red blood cells that have been separated to be used as a blood transfusion in patients (5).

2.2 Epidemiology

The incidence of NEC is estimated to range from 0.4 and 1.8 in 1000 live births (6)(7). This incidence increases with a decrease in birth weight, being as high as 11% in VLBW infants (8).

NEC associated mortality ranges between 15 – 30%, with birth weights and mortality rates being inversely proportionate (9)(10)(11). There is no definitive prevention or cure for NEC as yet, and approximately 35 - 50% of patients with NEC will require surgical intervention, whereas the rest are managed medically(12)(10)(13). Mortality, as well as long term sequalae, such as neurodevelopmental delay, growth retardation and chronic gastrointestinal complications, are increased in patients who require surgical intervention(14).

Most neonates who develop NEC are born preterm, but it rarely also occurs in term neonates. For the purpose of this study we will be focussing specifically on preterm neonates who developed NEC, as the pathophysiology and aetiology of NEC in term neonates are thought to differ.

NEC also poses a large social-economic burden, leading to considerably longer hospitalisation times. In one study this translates into as much as 60 days longer length of stay for neonates with NEC who required surgical intervention (15).

2.3 Pathophysiology

Active research is ongoing for a more complete understanding of the aetiology and pathophysiology of NEC. At tissue and cellular level, NEC seems to be an inflammatory process, with overgrowth of bacteria, and necrotic changes. Worsening of the

aforementioned pathological processes, can lead to air in the intestinal wall (pneumatosis intestinalis), air in the portal venous and lymphatic systems, and complete disruption of the intestinal wall leading to intestinal perforation and pneumoperitoneum (4).

The singe common denominator seems to be the presence of an excessive inflammatory response (probably due to an immature intestinal immunity) to a yet ill-defined insult. Some of the postulated possible causes for this response includes overgrowth of normal bacteria, ischemia, infection due to abnormally localized bacteria, and different feeding strategies (16).

2.4 Diagnosis

Recognition of the clinical signs of NEC is of great importance to any doctor working with neonates, due to the possibility of a rapid and fatal progression of this disease. Early signs are very non-specific, and can include apnoea, changes in baseline heartrate, changes in level of consciousness, and temperature instability. Signs implicating the gastro-intestinal tract include feeding intolerance, blood per rectum, and abdominal distention. Later, abdominal tenderness, discoloration of the abdominal wall, and a palpable abdominal mass, will indicate progression of the disease process (12).

However, to confirm the presence of NEC, radiographic evidence is required. A combination of clinical and radiological evidence makes scoring the severity of the insult possible, by making use of the Modified Bell's Score (Table 1) (1). Laboratory studies usually reveal a nonspecific inflammatory process. Currently there is no specific test available to confirm NEC, or predict the course thereof.

STAGE	CLINICAL FINDING	RADIOGRAPHIC FINDINGS	GASTROINTESTINAL FINDINGS
I: Suspected	Apnoea Bradycardia Temperature instability	Suggestive of Ileus only	Increased NG Aspirates Occult Blood in Stool Mild Abdominal Distention
IIa: Definite	Apnoea Bradycardia Temperature instability	Ileus with dilated loops Focal pneumatosis	Macroscopic blood in stool Severe abdominal distention

			Absent bowel sounds
IIb	Thrombocytopenia Mild metabolic Acidosis	Widespread pneumatosis Ascites Portal Venous Gas	Abdominal Wall Oedema Tenderness Palpable Bowel
IIIa: Advanced	Mixed acidosis Oliguria Hypotension Coagulopathy	Prominent bowel loops Worsening Ascites No free air	Worsening wall oedema Erythema Induration
IIIb	Shock Deteriorating Laboratory values and Vital Signs	Pneumoperitoneum	Perforated bowel

Table 1: Modified Bell's Staging for Necrotising Enterocolitis (1)

Plain radiography remains the mainstay of imaging in NEC patients, due to its reliability in exhibiting the necessary manifestations. These include, in order of worsening severity, a typical ileus bowel gas pattern, thickened bowel walls, pneumatosis intestinalis (gas in the bowel walls), portal venous gas, and pneumoperitoneum (indicative of hollow viscus perforation). Another imaging modality that can be utilized in the diagnosis and management of NEC is abdominal ultrasonography. The additional use of colour duplex Doppler imaging can assist in evaluating the thickness of the bowel wall, echogenicity thereof, fluid collections, peristalsis, pneumatosis too small to be visible on plain films, and appraise the quality of bowel wall perfusion (12).

2.5 Prevention

Previously the focus was solely on the management of NEC once it has already developed. This unfortunately did not lead to any significant improvement in the outcome or long term complications. Currently the focus of research has moved more towards the prevention of NEC development in the first place. Thus far the main focus in prevention of NEC has been adjustments in feeding strategies and gastro-intestinal flora.

Where feeds are concerned, the time of initiation of feeds, type of feeds (breastmilk versus formula feeds), and rate of escalation of feeds, are important variables. Initiating early feeds in preterm infants has been thought to increase the risk for developing NEC, although

a systemic review by Kennedy and Tyson in 2000 concluded that the available studies had a too small sample size to adequately back this finding (17). Neonates who are breastfed have been found to have a lower risk of developing NEC than those who receive formula feeds, despite the age at onset of feeds (18)(19). Although some studies have suggested that a higher rate of advancement of feeds once initiated, carries a higher risk for developing NEC (20), the results from this study is still being scrutinized. Current feeding strategies at the NICU's in Pelonomi and Universitas Hospital include delaying feeds in preterm neonates, and advocating for breastmilk feeds rather than formula feeds whenever possible.

In addition to changes in feeding strategies, the use of enteral antibiotics and probiotics has been suggested to protect against NEC, but this is still controversial and is not currently being utilized at any of our local units.

2.6 Management

The management of NEC depends on the clinical condition of the neonate, with surgical intervention only being utilized once necrosis or subsequent perforation of the bowel is present. Initial treatment is aimed at general patient support, and finding and reversing the causative factor. Thus a septic work-up is done on all neonates showing signs of NEC, after which empirical antibiotic therapy will be commenced. All enteral feeds are discontinued, and a nasogastric tube is inserted to decompress the stomach and proximal bowel. Parenteral feeds and fluid resuscitation is also started (16).

Some controversy still exists regarding the exact timing of surgical intervention, but the most commonly used clinical parameters utilized includes bowel perforation (distinguished by pneumoperitoneum on abdominal x-rays, or the finding of bowel content on paracentesis) (1). Surgical intervention ranges from primary peritoneal, to laparotomy and resection of necrotic bowel.

2.7 Transfusion Associated NEC (TANEC)

The association between transfusion of blood products and the development of NEC in preterm neonates was first described in 1987 by McGrady et al. (21), although very little further research was conducted into this phenomenon until the last decade. This has led to larger neonatal centres reviewing their transfusion strategies, as well as adopting

protocols where at risk neonates do not receive enteral feeds immediately before and after receiving a transfusion of blood products. There is however still wide spread controversy regarding this topic, with some studies showing no significant correlation between transfusion of PRBC's and the development of NEC (22).

Transfusion associated necrotising enterocolitis, also called transfusion related acute gut injury (TRAGI) in some units, is thought to be an adverse reaction to blood transfusions, similar to the pulmonary counterpart found in adults, namely "transfusion related lung injury" (TRALI) (3). This pathological process is hypothesized to be due to a multifactorial insult on the intestinal barrier. Firstly, at cellular level, there is thought to be an immunological response to Human Leucocyte Antigen (HLA) and other antigens present in the transfused blood. This leads to a cascade of endothelial cell activation (5). This immune reaction, in combination with the viscosity of the blood products, is thought to lead to a decrease in mesenteric blood flow directly after transfusions, and enteral feeds in the presence of decreased mesenteric blood flow can lead to the development of NEC (23). The last factor influencing the perfusion state of the bowel after a blood transfusion, can be the age of the donor blood, leading to a decrease in capacity to deliver oxygen to tissues, and subsequently leading to vasoconstriction and ischemia.

The neonatal population receive some of the highest amounts of blood transfusions in hospitals. However, very few guidelines exist regarding the threshold for transfusions in neonatal units. Even where guidelines do exist, it is seldom adhered to. This is mostly due to the previous school of thought being that to optimize the oxygen delivery to tissues, we must optimize the haemoglobin delivery system by transfusing packed cells. However, recent studies have shown that unnecessary transfusions of packed red blood cells (PRBC's) have more detrimental effects in the neonatal population than previously thought, and that oxygen delivery to peripheral tissues may be impaired at too high an artificial haematocrit. This has led to more scrutiny in the practice of PRBC transfusions.

The most commonly used guideline for transfusion of PRBC's in neonates, are as set out in Table 2 (24).

Indications for Transfusion or PRBC's in Neonates	
HCT	**AGE and CLINICAL CONDITION**
<35%	≤1week of Life **AND** Unstable*
<28%	≤1week of Life **OR** Unstable*
<20%	>1week of Life **AND** Unstable*

*Unstable clinical condition is defined as a state of poor oxygen delivery, e.g. Respiratory distress/apnoea, Cardiovascular deterioration etc.

Table 2 : Indications for Transfusion or PRBC's in Neonates (24)

The impact of enteral feeds on mesenteric blood flow, and oxygen delivery, during PRBC transfusions, is of great importance. By making use of near infrared spectroscopy in measuring the mesenteric regional oxygen saturation in neonates receiving transfusions, Marin et al. showed a decrease in mesenteric oxygenation in patients receiving concurrent feeds, as opposed to an increase in patients in whom enteral feeds were halted during the transfusion (23).

3. Problem Statement

Is there an association between Red Cell Concentrate Transfusions in Very Low Birth Weight Neonates, and the development of NEC, as well as the severity of the NEC that develops?

4. Aims and Objectives

To evaluate the transfusion practices in two Neonatal Units in Bloemfontein, Free State, and determine whether an association exists between transfusions in neonate, the time frame of enteral feeds in relation to the transfusions, and the development of NEC. To compare the transfusion practices between the two hospitals, over a 5-year period, and to evaluate possible differences in outcomes, specifically pertaining to NEC.

5. Methodology

5.1 Study Design

An analytical retrospective review

5.2 Inclusion Criteria

All Very Low Birth Weight (1000g – 1499g) neonates born in the Pelonomi Hospital and Universitas Academic Hospital, and admitted to their neonatal units, in a 5-year period. The time frame will be between January 2012 and December 2017.

The expected number of patients included will be between 1000 and 1200.

5.3 Exclusion Criteria

- Neonates born in other hospitals, and admitted after 48hours of life.
- Any other congenital surgical conditions.
- Demise within 72 hours of life.

5.4 Measurement

Patients will be identified by making use of the statistics of the Department of Paediatrics and Child Health. Further information will be obtained from the Meditech database, and patient files. This information will include:

- Severity of NEC (Bell Score)
- Whether the NEC presented within 48hours of a transfusion
- Whether NEC was present before the transfusion
- Whether the patient was kept nil per os (NPO) during and after the transfusion
- Whether the patient was concurrently ventilated
- The patient's haematocrit before, and after the transfusion

This information will be recorded on a data form. (Appendix C)

5.5 Limitations

- Possible errors in compiling of the Department of Paediatrics and Child Health statistics, can lead to patients being accidentally left out of the study.
- Incomplete patient notes could also lead to possible errors in data.

6. Analysis

Data will be collected on the data forms, and subsequently typed into a Microsoft Excel spreadsheet by the researcher. The data will then be analysed by the Department of Biostatistics at the University of the Free State. Results will be summarised by frequencies and percentages (categorical variables) and means, standard deviations or percentiles (numerical variables). Associations will be assessed by relative risks with 95% confidence intervals, and appropriate hypothesis testing.

6.1 Pilot Study

A brief pilot study will be conducted, be evaluating the first 20 patients found by above mentioned selection methods, and thereby evaluating the adequacy of the data form in use (Appendix C). Afterwards these 20 patients, and their data, will be included in the final study.

6.2 Ethical Considerations

No details that will make it possible to identify patients, or their family members, will be used on the data collection forms. All precautions will be taken by the researcher to ensure anonymity of participants and their personal information. Approval will be obtained from the Health Sciences Research Ethics Committee of the University of the Free Sate. Permission to conduct the study will also be obtained from the Department of Health of the Free State.

6.3 Budget and Time Schedule

BUDGET

Printing Paper	R100
Ink Cartridge	R500
Total	**R600**

(Paid by the Department of Surgery)

TIME SCHEDULE

MAY 2017	Hand in Protocol for Ethics Committee Clearance
JUNE 2017	Clearance from Free State Department of Health
AUG – DEC 2017	Collect and Capture Data
JAN – FEB 2018	Analysis by Department Biostatistics
MARCH – MAY 2018	Write up of Dissertation

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Appendix E

Transfusion Practices in Very Low Birth Weigh Neonates, and the Development of Necrotising Enterocolitis, in two Neonatal Units in Bloemfontein, Freestate.

O O

For office use

Subject nr: | | | | | 1-4

1. Demographic data

1.1 Date of Birth: _____/_____/_____ (dd mm yyyy) | 5 - 12

1.2 Birth Weight _____ g | 13 - 16

1.3 Gestation in Weeks: _____ weeks (Best estimate) | 17 - 18

2. Transfusion Data

2.1 Pre-transfusion Haematocrit
_____ L/L | 19 - 21

2.2 Post-transfusion Haematocrit
_____ L/L | 22 - 24

2.3 NPO during transfusion? | 25

YES
NO

2.3 Time NPO after transfusion?

_____ min | 26 - 28

2.4 Ventilatory Support during transfusion | 29

HFOV
IPPV
NCPAP
NHFO
RA

3. NEC Data

3.1 NEC Developed? | 30

NO
After (48hr)
Before TF

3.2 Severity of NEC: (Bell Score) | 31

I
II (a)
II (b)
III (a)
III (b)

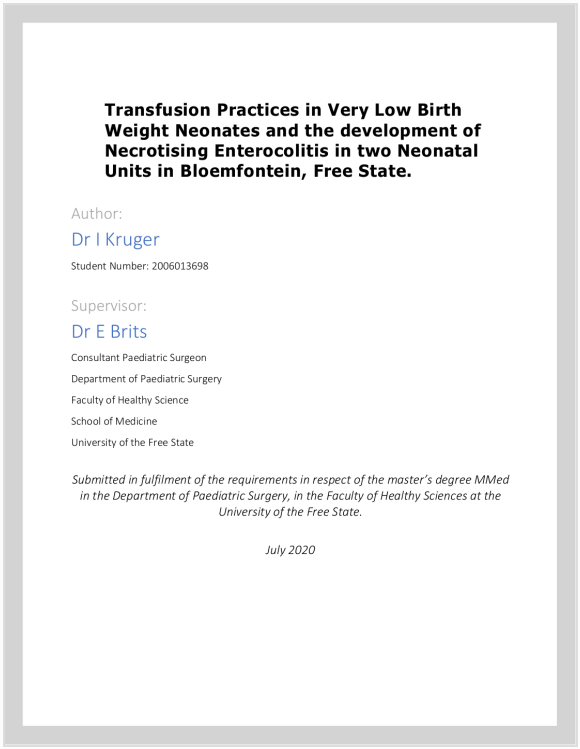
Appendix F

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Cassandra Ferreira
Assignment title:	Draft Box
Submission title:	Transfusion Practices in Very Low B…
File name:	TANEC_10.pdf
File size:	658.7K
Page count:	77
Word count:	22,227
Character count:	123,562
Submission date:	22-Sep-2020 11:29AM (UTC+0200)
Submission ID:	1359891248



Transfusion Practices in Very Low Birth Weight Neonates and the development of Necrotising Enterocolitis in two Neonatal Units in Bloemfontein, Free State.

Author:
Dr I Kruger
Student Number: 2006013698

Supervisor:
Dr E Brits
Consultant Paediatric Surgeon
Department of Paediatric Surgery
Faculty of Healthy Science
School of Medicine
University of the Free State

Submitted in fulfilment of the requirements in respect of the master's degree MMed in the Department of Paediatric Surgery, in the Faculty of Healthy Sciences at the University of the Free State.

July 2020

Appendix G

Transfusion Practices in Very Low Birth Weight Neonates and the development of Necrotising Enterocolitis in two Neonatal Units in Bloemfontein, Free State. Author: Dr I Kruger

by Cassandra Ferreira

Submission date: 22-Sep-2020 11:29AM (UTC+0200)
Submission ID: 1359891248
File name: TANEC_10.pdf (658.7K)
Word count: 22227
Character count: 123562

Transfusion Practices in Very Low Birth Weight Neonates and the development of Necrotising Enterocolitis in two Neonatal Units in Bloemfontein, Free State.

Author:

Dr I Kruger

Student Number: 2006013698

Supervisor:

Dr E Brits

Consultant Paediatric Surgeon

Department of Paediatric Surgery

Faculty of Healthy Science

School of Medicine

University of the Free State

Submitted in fulfilment of the requirements in respect of the master's degree MMed in the Department of Paediatric Surgery, in the Faculty of Healthy Sciences at the University of the Free State.

July 2020

Declaration

I, Iselma Kruger, hereby declare that the minor dissertation titled "Transfusion Practices in Very Low Birth Weight Neonates and the Development of Necrotising Enterocolitis in two Neonatal Units in Bloemfontein, Free State", is my own work and has not been submitted previously by me to this, or any other tertiary institution. I furthermore declare that all sources which I have used or quoted, have been indicated and acknowledged with complete references.

_____

Dr I Kruger

September 2020

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I would like to acknowledge the following contributors, who assisted in the completion of this study.

- Dr E Brits, my supervisor, for her insight and guidance in this endeavour.
- Prof G Joubert, for her assistance, and endless patience, regarding the statistical analysis of the data.
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- Dr MA Pienaar for his input with ideas and brainstorming in the beginning of this project.

I would also like to acknowledge the following heads of department for allowing me access to their patient's information.

- Dr SM le Grange, Head of Paediatric Surgery, University of the Free State.
- Dr T Mosia, Head of Neonatal Units at Pelonomi Tertiary Hospital and Universitas Academic Hospital.

This dissertation is dedicated to my mother, Dr Elize Kruger, for always being a pillar of support and inspiration.

Table of Contents

Abstract:

Background:

Necrotising enterocolitis (NEC) places a massive burden on neonatal units and the healthcare system. Not only does it significantly prolong the hospital stay of neonates, it also causes detrimental long-term sequelae, such as neurodevelopmental delay, growth retardation and chronic gastrointestinal complications. However, despite it being a well-documented disease, with the first reports of clinical findings indicative of NEC being made as early as the early 1800's, it continues to elude neonatologists in terms of risk factors, prevention and treatment strategies.

Further research is necessary to help identify possible contributing factors, to improve treatment, as well as to develop preventative strategies for this difficult condition. One possible contributing factor that has been identified by researchers, is the transfusion of blood products in the neonatal period, especially in preterm neonates. Since there are currently no standardised Red Blood Cell (RBC) transfusion protocols in either of the two Neonatal Intensive Care Units (NICU's) in Pelonomi Tertiary Hospital (PTH), and Universitas Academic Hospital (UAH), patients receive RBC transfusions at a great variety of different clinical stages.

Objectives:

By retrospectively evaluating the RBC transfusion practices in these units, specifically in those patients who developed NEC, we hoped to gain better insight into the possible causative relationship between RBC transfusions, and the development of NEC in Very Low Birth Weight (VLBW) neonates.

Method:

Data was collected on a total of 1585 VLBW neonates who were treated at PTH, and UAH, during a retrospective 5-year period. Data collected included gestational age, birth weight, RBC transfusion data, and data regarding the development of NEC. The RBC transfusion data included their pre-transfusion haematocrit, post-transfusion haematocrit, recorded clinical state during transfusion, ventilatory state during transfusion, and whether they were kept nil per os (NPO) during, and for at least 120minutes after the transfusion.

Regarding NEC, for all VLBW neonates who developed NEC the following data was recorded: The Modified Bell's Staging grade, whether they developed NEC before an RBC transfusion, or within 48-hours after an RBC transfusion.

Results:

This study showed that the incidence of NEC in VLBW neonates in these two academic hospitals was higher than the expected international number. There was a definite decrease in the amount of VLBW neonates who received RBC transfusions over the 5-year period, with an improvement in feeding practices during transfusions. This decrease in RBC transfusions correlated with a decrease in the incidence of TANEC, but no statistical significance between

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feeding practices during RBC transfusion and the development of TANEC, could be demonstrated.

Keywords

Definition of Terms:

Neonate:

Any new-born baby up to the age of 28 days.[1]

Preterm Neonate:

Any neonate born to a mother who has been pregnant for less than 37 weeks.[1]

Necrotising Enterocolitis (NEC):

Necrotising enterocolitis is necro-inflammatory cellular death (necrosis) of the bowel of a neonate, diagnosed by utilizing clinical and radiographic findings, as described in a later section (Diagnosis).

Transfusion Associated Necrotising Enterocolitis (TANEC):

NEC that occurs in a patient within 48-hours after receiving a transfusion of red blood cells.[2]

Very Low Birth Weight Neonates (VLBW):

Any neonate born at a weight of between 1000g and 1499g.[2]

Extremely Low Birth Weight Neonates (ELBW)

Any neonate born at a weight of <1000g.[2]

Enteral Feeds:

Nutritional substances (feeds) delivered to the patient directly via the gastro-intestinal tract (thus either via the mouth, or directly into the stomach or small intestine).[2]

Red Blood Cells (RBC's):

Donor red blood cells that have been separated to be used as a blood transfusion in patients.[3]

Exchange Transfusion:

A procedure where the patient's blood is slowly removed and simultaneously replaced with donor blood. All of the patient's blood is replaced in this way to removed harmful substances such as high levels of bilirubin.[4]

List of Abbreviations

NEC:	Necrotising Enterocolitis
VLBW:	Very Low Birth Weight (as pertaining to a neonate)
TANEC:	Transfusion Associated Necrotising Enterocolitis
RBC's:	Red Blood Cell's (as pertaining to a transfusion)
PTH:	Pelonomi Tertiary Hospital
UAH:	Universitas Academic Hospital
NPO:	nil per os
ICU:	Intensive care unit
NICU:	Neonatal intensive care unit
PAF:	Platelet activating factor
TNF:	Tumour necrosis factor
IPPV:	Intermittent positive-pressure ventilation
HFOV:	High-frequency oscillatory ventilation
NHFO:	Nasal high-flow oxygen
NCPAP:	Nasal continuous positive airway pressure
DIC:	Disseminated intravascular coagulation
EGF:	Epidermal growth factor

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B. Free state Department of Health (FSDoH) Approval Letter
C. Head of Department (HoD) Approval Letter
D. Research Protocol
E. Data form
F. Turnitin digital receipt
G. Turnitin similarity report

Chapter 1: Introduction

1.1 Motivation for this study

Necrotising enterocolitis (NEC) places a massive burden on neonatal units and the healthcare system. Not only does it significantly prolong the hospital stay of neonates, it also causes detrimental long-term sequelae, such as neurodevelopmental delay, growth retardation and chronic gastrointestinal complications[1]. However, despite it being a well-documented disease, with the first reports of clinical findings indicative of NEC having been made as early as the early 1800's [5], it continues to elude neonatologists in terms of risk factors, prevention and treatment strategies. This paucity of evidence is a great motivation for this study.

Further research is necessary to help identify possible contributing factors, to develop more accurate preventative strategies, as well as to help implement treatment regimes. Multiple risk factors have been postulated, with some proven, and some still speculative. One possible contributing factor that has been identified by researchers, is the transfusion of blood products in the neonatal period, especially in preterm neonates. Enteral feeding during the transfusion of blood products has also been identified as a significant modifiable risk factor[6]. Since there are currently no standardised RBC transfusion protocols in the two Neonatal Intensive Care Units (NICU's) in PTH, and UAH, patients receive RBC transfusions at a great variety of different clinical stages, and not all are kept NPO during and for an adequate time after their transfusions.

By retrospectively evaluating the RBC transfusion practices in these units, specifically in those patients who had developed NEC, we hoped to gain better insight into the possible causative relationship between RBC transfusions and the development of NEC. By evaluating the transfusion practices over an adequate span of time, we also hoped to identify the impact of changes in these transfusion practices and help create a scientific basis for the development of a standardized transfusion protocol in these units.

1.2 Research Purpose

1.2.1 Primary Objectives

The aim of this study is to evaluate the RBC transfusion practices in VLBW neonates in two academic neonatal units in Bloemfontein, Free State, in correlation with International Guidelines by:

- Comparing and describing the RBC transfusion practices between the two hospitals
- Recording any changes in RBC transfusion practices, over a 5-year period
- Evaluating possible differences in outcomes, specifically pertaining to NEC

1.2.2 Secondary Objectives

Additionally, we aim to gain further insight into the impact of RBC transfusion practices in VLBW neonates by evaluating:

- The haematological indications for RBC transfusions
- The clinical state of neonates during RBC transfusions
- Whether or not they were receiving enteral feeds during, and for 120min after, the RBC transfusion
- The possible association between the RBC transfusions, the timing of enteral feed in relation to the RBC transfusion, and the development of NEC as well as the severity thereof

By evaluating our practices over a 5-year period and being able to identify possible shortcomings in our RBC transfusion protocols, we hope to significantly improve our clinical practice.

1.3 Structure of this dissertation

Chapter 1 serves to introduce readers to the study in context of the current literature whilst outlining the aims, objectives, and potential contributions to the field.

Chapter 2 presents a concise, yet thorough review of the important literature published regarding this topic as well as internationally accepted practices. A definition of the most important terms, epidemiological review, description of the pathophysiology, methods for making the diagnosis, preventative strategies and management strategies will be discussed. A brief description of Transfusion Associated Necrotising Enterocolitis (TANEC) and the theories regarding this is also discussed as background and motivation for this study.

Chapter 3 will describe the research question and outline the problem statement and aim of this study.

Chapter 4 outlines the specifics regarding the research design of the study, methods used for data collection, statistical analysis, and ethical considerations and approval received.

Chapter 5 presents all the results obtained from the data collected, outlining specifically the general epidemiological data, the RBC transfusion results, and NEC results for both PTH and UAH separately, and a comparison between the two institutions. A review of the RBC transfusion practices as evaluated over 5 years, as well as the incidence of NEC over the 5 years is also compared.

Chapter 6 is a brief discussion of the results in the context of the latest literature, comparing the results of our study to results from other studies around the world.

Chapter 7 will lastly aim to bring the results as outlined in chapter 4, into context with the current literature, present the final conclusions of the study and delineate potential areas of future research.

Chapter 2: Literature Review

2.2 Introduction

Although NEC as a disease entity has been well known and documented since the early 1950's, it is still a clinical conundrum.[7] Since it has proven to be one of the most common and deadliest surgical emergencies in the neonatal population, large volume research has been conducted in the past couple of decades.[8] Despite this large volume research, no definite cause has been identified yet, and the need for further research remains.

This chapter will present the current available literature on NEC, and the potential link between NEC and RBC transfusions. The aim of this chapter is to provide some in-depth background information regarding these topics, and to provide the groundwork of knowledge for understanding this study, as well as for implementation of the results.

2.3 Epidemiology

The incidence of NEC is estimated to range from 0.4 to 1.8 in 1000 live births.[9,10] This incidence is indirectly proportional to birthweight, increasing with a decrease in birth weight, being as high as 11% in VLBW neonates.[11] However, the incidence of NEC seems to vary greatly between different institutions and countries. Specifically, traditionally thought to be of importance was the difference between low to mid income countries, and high-income countries. The National Institute of Child Health and Human Development recorded a variation of between 4% and 20% at institutions across the USA.[12]

A more recent systematic review conducted by Alsaied et al, estimated the incidence of NEC in VLBW neonates to be 7%. They reviewed 27 cohort studies that reported the incidence of NEC, from both low to mid income countries, and high-income counties. There was no statistically significant difference recorded in the incidence of NEC between the different income countries, but a marked increase in the incidence of NEC was noted over the years. They also suggested that more studies in low to mid income countries are needed, as a lack of research in these countries may lead to an underestimation of the incidence.[13]

Since the term "necrotising enterocolitis" was first used in 1953 by Schmid and Quaiser,[7] there has been a vast increase in the number of neonates diagnosed with this condition. This increase in incidence is attributed both to the increased number of premature babies delivered via Caesarean section, as well as the improvement in neonatal intensive care, ensuring the survival of more premature and lower birth weight neonates, who are at a higher risk for developing NEC.[12]

NEC associated mortality ranges between 15 and 30%, with birth weights and mortality rates being inversely proportional.[14,15,16] There is no proven definitive prevention or cure for NEC as yet, and approximately 35 - 50% of patients with NEC will require surgical intervention, while the rest can be managed medically.[17,15,18] Mortality, as well as long term sequalae such as

neurodevelopmental delay, growth retardation and chronic gastrointestinal complications are increased in patients who require surgical intervention.[19]

Most neonates who develop NEC are born preterm, but it sometimes also occurs in term neonates, with up to 12% of all NEC cases occurring in the latter group.[12] For the purpose of this study we will be focussing specifically on preterm neonates who developed NEC, as the pathophysiology and aetiology of NEC in term neonates are thought to be different.

In addition, NEC poses a large social-economic burden leading to considerably longer hospitalisation times, increased need for intensive care unit (ICU) care, and increased need for assisted ventilation. In one study this translates into as much as 60 days longer length of stay for neonates with NEC who required surgical intervention.[20] Especially in lower income countries this is a heavy economic burden to bare.

2.3 Pathophysiology

Active research is ongoing to gain a more complete understanding of the aetiology and pathophysiology of NEC. At tissue and cellular level, NEC seems to be an inflammatory process, with overgrowth of bacteria, and necrotic changes, but the exact order in which these pathological processes take place is still unknown. Worsening of the aforementioned pathological processes, can lead to air in the subserosal and submucosal layers of the intestinal wall (pneumatosis intestinalis), air in the portal venous and lymphatic systems, and complete disruption of the intestinal wall leading to intestinal perforation and pneumoperitoneum.[2] The pneumatosis is due to nitrogen and hydrogen produced by gas producing bacteria in the gastro-intestinal tract, with the gas penetrating the intestinal to become radiologically evident.[21] Histologically the bowel of neonates with developing, or early NEC will show microthrombus formation with resultant transmural oedema, patchy ulceration, and haemorrhage.[22]

The most prominent single common denominator seems to be the presence of an excessive inflammatory response to a yet ill-defined insult. This excessive inflammatory response it thought to be due to immature intestinal immunity. Some of the postulated insults or causes for this response includes overgrowth of normal bacteria, ischemia, infection due to abnormally localized bacteria, and different feeding strategies.[23]

2.3.1 Ischemic changes

Intestinal circulation is controlled by both extrinsic and intrinsic factors. The autonomic nervous system and cardiovascular reflexes extrinsically control splanchnic blood flow, by diverting blood flow away from the intestinal circulation in an attempt to preserve systemic perfusion. In addition, the intestines form different vasoactive mediators that have an intrinsic control over the local blood flow.[24]

Resting vascular resistance is the biggest factor that influences intestinal microcirculation. As per the Hagen-Poiseuille equation the rate of flow in a vessel is proportional to the radius of the vessel to the fourth power. This means that small changes in vascular diameter, due to vasodilatation or vasoconstriction, leads to much larger changes in vascular resistance and blood flow.[24] As mentioned the autonomic nervous system and cardiovascular reflexes will divert blood away from the splanchnic system if there are any changes in the systemic blood flow that necessitate perfusion of vital organs such as the heart and kidneys. This hypothesis

thus means that the resultant vasoconstriction could severely impair the perfusion of the intestines.

The correlation between changes in intestinal circulation and intestinal injury has been under investigation for more than four decades. The first accepted hypothesis was that perinatal asphyxia leads to decreased intestinal blood supply, due to the extrinsic neurogenic blood flow redistribution. Premature neonates delivered due to obstetric complications such as foetal distress, tend to suffer from birth asphyxia and have lower Apgar scores at birth. This in turn would lead to intestinal mucosal ischemia. However, the fact that NEC very rarely develops in the first seven days of life in preterm neonates, disproves this hypothesis.[16] Also of note is the fact that sustained adrenergic stimulation has not been proven to cause any sustained intestinal hypoxia.[25]

Other causes of neonatal hypoxic stress have also been scrutinized. The following list includes other conditions or interventions that could lead to hypoxic intestinal hypoperfusion, and potentially lead to the development of NEC, although none of them have been conclusively proven as a sole cause for NEC:

- Sepsis
- Respiratory distress syndrome
- Cyanotic congenital heart disease
- Umbilical vessel catheterization
- Patent ductus arteriosus
- Polycythaemia[26]

The intestinal blood supply flows from the serosa on the outside of the bowel to the mucosa on the inside. Thus, the first area where ischemia will become apparent during decreased perfusion, is in the internal mucosal layer.[8] This accounts for the fact that ischemia and necrosis is histologically first visible in the mucosal layer, and then spreads to eventually involve the entire intestinal wall.

The last thing to consider when looking at hypoxic stress in neonates are vasoactive substances in the intestinal microcirculation, thereby intrinsically controlling intestinal perfusion. Multiple vasodilators and vasoconstrictors play an important role in balancing blood flow to the intestine. Two of these important vasoactive substances include endothelin-1 and endothelial nitric oxide (NO), imbalances in which during pathological states can lead to ischemia in the intestinal wall.[8]

The endothelium of the intestinal microvasculature plays an important role in intestinal perfusion by regulating these intrinsic vasoactive peptides. Endothelin-1 is responsible for vasoconstriction, and nitric oxide for vasodilatation, and the balance of these peptides are important for continued adequate blood flow in the microvasculature of the intestinal wall. The inflammatory cascade can lead to imbalances in these two peptides by increased production of endothelin-1, leading to excessive vasoconstriction and impaired intestinal perfusion.[27]

The role of intestinal microcirculation in the ischemic changes seen in NEC is well known, but it is still unclear whether the ischemia is the cause of the NEC, or rather a consequence of the disease process.

2.3.2 Intestinal microbiota

Most VLBW neonates are cared for in an ICU or high care unit for the first few days to weeks of their life. This contrasts with healthy term neonates who are immediately handed over to the care of their mothers, where maternal contact and breastfeeding is encouraged. Close contact between mother and baby promotes the transfer of intestinal microflora, which is thought to improve nutrition and strengthen the intestine's epithelial barrier.[28] The normal microbiota is important in the immunity of the gut. By stimulation of the Paneth cells to secrete peptides, they promote an environment that resists the growth of pathogenic organisms.[29]

Preterm, VLBW neonates frequently end up being formula fed due to the mothers often having difficulty expressing their breastmilk, and the challenge of producing adequate quantities of breastmilk without the direct stimulation of the baby. NICU's and high care units also do not allow for as much direct maternal contact. Other risk factors for abnormal gut flora includes hygiene practices in the units, unnecessary and prolonged use of antibiotics, stasis of bowel content or reduced bowel motility, prolonged parenteral nutrition, and birth via caesarean section.[28] The dysbiosis of neonates delivered via caesarean section, and the importance there-of, has been an ongoing dispute for the past decade. Even though some studies have proven that neonates born via caesarean section had a different intestinal microbiome from babies delivered via normal vaginal delivery (NVD), the importance and clinical significance of these findings are still uncertain, and thus we did not make use of this parameter in our study.

Neonates who are cared for in ICU and high care units are also more frequently exposed to nosocomial pathogens that are proven to be more resistant and virulent in their effects. These pathogens are thought to be the cause of Late Onset Neonatal Sepsis (LOS) and NEC in VLBW neonates. This theory is supported by the fact that most preterm neonates only develop NEC after the first week of life, as well as by the outbreak of clusters of NEC in certain units.[8]

A recent study published by Brehin et al in July 2020 suggested that the use of aminoglycosides (e.g. Gentamycin) and glycopeptide antibiotics (e.g. Vancomycin) could lead to bacteria that are resistant and an increased incidence in NEC in preterm neonates. The use of aminoglycosides in the first week of life can also hamper normal bacterial colonization, as they impair the growth of Proteobacteria, and lead to the overgrowth of pathogens.[30]

Some of the pathogens that have been isolated in neonates with NEC include:

- *Escherichia coli*
- *Enterobacter sakazakii* and other species
- *Staphylococcus epidermidis*
- *Klesbsiella sp.*

- *Streptococcus sp.*
- *Clostridium sp.*[31,30]

2.3.3 Feeding and mechanical injury

In a 2009 Case Control Study, Henderson et. al showed that the rate of increase in feeding volumes is one of the important proven modifiable risk factors for the development of NEC in VLBW neonates. None of the neonates they evaluated had developed NEC before enteral feeds were commenced, but the neonates who developed NEC had a significantly faster advancement in feed rate than the control group who did not develop NEC. They also found a significantly lower incidence of NEC in the neonates who were exclusively breastfed, versus the neonate who were formula fed, or received mixed feeds.[32]

The mechanisms by which differences in volumes and rates of enteral feeds influence the development of NEC is however still not well understood. The leading theory is that it is due to disruption of the intestinal mucosal integrity, intestinal blood flow, and intestinal motility.[29] One of the differences between the intestine of term and preterm neonates is thought to be the mucus layer in the intestine preventing bacterial translocation. This layer is underdeveloped in the premature intestinal tract with discontinuations which can lead to trauma of the intestinal mucosa and bacterial translocation.[28]

During the third trimester, the foetus ingests amniotic fluid, which is rich in nutrients and growth factors, leading to intestinal development. The newborn infant then ingests breastmilk, which is also advantageous for intestinal development due to its complex composition of nutrients, immunomodulators, and hormones. This combination of amniotic fluid and breastmilk ingestion is thought to be the link to the low incidence of NEC in term breast-fed infants, whereas VLBW preterm neonates are born without their intestinal barrier having matured properly, rendering breastfeeding even more important in these infants.[33] However, even in breastfed VLBW neonates it is often impossible to advance their enteral feeds enough to achieve adequate levels of defence, and this has led to the addition of other substances such as probiotics, prebiotics and lactoferrin to promote intestinal immunity.[28]

A recent study by Harutuynyan et al compared neonates who received a multi-modal three-component enteral medication regime upon diagnosis of NEC, with neonates who did not. The medication included an antibiotic, an antifungal agent, and a probiotic. They found that the neonates who had received these enteral medications had a better outcome regarding mortality and the need for surgical intervention.[34] Nevertheless, as the data they used was only observational, this study could only generate a hypothesis, and further studies are indicated.

2.3.4 Immature intestinal barrier response

As discussed above, the intestine of a preterm neonate basically has the same make-up and qualities as the intestines of a foetus. This intestine is not yet adequately mature to deal with the same level of exposure and functionality as the intestinal tract of a term neonate. The mature intestine has both microbiota and physical barriers protecting it from bacterial translocation.[35]

The unique anatomical make-up of the intestinal barrier, a single epithelial layer that is continuously in contact with intestinal content, serves to maximize nutritional absorption. However, this quality can lead to ease of bacterial uptake as well, and thus the intestine needs special physical barrier defence mechanisms to prevent this.

These physical barriers include intestinal mucus production, peristaltic movement, cell surface glycoconjugates, and tight junctions between intestinal epithelial cells.[35] All of these barriers serve to limit the amount of bacteria present in the gut lumen, as well as preventing attachment of these bacteria to the intestinal epithelium, which could lead to bacterial translocation.

In premature neonates these physical barriers are poorly developed. For instance, the first intestinal layer that gets into contact with bacteria is the mucous layer of the gut that overlies the epithelial cells. This mucous layer has been found to be less dense and to have lower levels of secretory immunoglobulins (see 2.3.5 Immature intestinal inflammatory response) in the premature gut.[35] Some studies have also suggested that there can be a paucity of mucin producing goblet cells in some genetically predisposed neonates.[36]

The next layer of the physical intestinal barrier includes the Paneth cells in the terminal ileum, and the Lieberkühn crypts which plays an important role in healing of the intestinal mucosa once damaged.[28] Both of these have been found to be immature, impairing their functionality in premature neonates. This combination of the defective immune defence mechanisms, and inability to adequately heal vulnerable and damaged areas in the intestinal mucosa, can lead to bacterial translocation.[31]

Also, important to take note of in the pathophysiology of NEC in premature neonates, is the paucity of peristaltic activity. The gut motility of VLBW neonates is significantly lower than that of term neonates, leading to stasis of the bowel content. This leaves the immature intestinal mucosa in contact with bacteria as well as other potentially dangerous substances for prolonged periods of time, potentially leading to damage, bacterial overgrowth and translocation.[37]

2.3.5 Immature intestinal inflammatory response

In addition to the physical barriers, there is also a functional barrier that is supposed to limit the growth of those bacteria which manage to overcome the physical barriers. This consists of the immunological factors such as intestinal lymphocytes, salivary IgA, and biochemical factors such as gastric acid secretion and proteolytic enzymes. Neonatal levels of these bioactive substances are decreased, further leading to an increased risk of bacterial translocation and the subsequent development of NEC.[35]

The most important inflammatory mediators identified that is known to play a role in intestinal immunological maturity are:

- Platelet activating factor (PAF)
- Tumour necrosis factor (TNF)
- Interleukins (1, 3, 6 and 8)
- Epidermal growth factor (EGF)

- Nitric oxide (NO)

PAF is a phospholipid inter and intra-cellular inflammatory mediator that causes intestinal epithelial cell apoptosis, but the exact mechanism of this intestinal injury is still under investigation. Patients with inflammatory bowel disease have been shown to have elevated serum and intestinal PAF levels, and in patients with NEC the PAF levels have correlated with disease severity.[35] In 2001 Rabinowitz et al found increased PAF levels in neonates several days before NEC became clinically evident. This can indicate that the raised serum PAF is an important initiating factor in the development of NEC.[38] Further support for this theory is the reduced incidence of NEC found in rats who received a PAF-degrading enzyme (PAF acetyl hydrolase).[39] Other than the direct inflammatory effect of raised PAF levels, it also initiates the increased production of other inflammatory mediators such as TNF, prostaglandins, complement and thromboxane.[40]

This inflammatory cascade, which is initially triggered by cell damage and apoptosis, will eventually lead to intestinal necrosis if uninhibited. To prevent this, intestinal enterocytes keep intestinal inflammatory responsiveness under control. In premature neonates the enterocytes have not adequately matured yet by the time that the intestinal epithelium is exposed to exogenous bacteria, and this can lead to an exaggerated inflammatory response. Compared to adult intestinal epithelial cells, foetal intestinal epithelial cells produce much more interleukin 8 (IL-8). IL-8 is a chemo-attractant cytokine that draws neutrophils into tissues, activate them, and alter their adhesion molecules.[35]

2.4 Risk factors

Although no exact cause for NEC has been identified yet, multiple risk factors have been identified that predisposes neonates to the development thereof. However, even identifying these risk factors is only one step in potentially preventing the development of NEC. Once these risk factors have been identified, implementation of preventative strategies have proven cumbersome, and impractical in some units.

2.4.1 Prematurity and intrauterine growth restriction

Even though NEC very rarely also occurs in term neonates, the vast majority of neonates who develop NEC are premature, and of low birth weight. Term neonates also tend to develop NEC earlier than their premature counterparts and have more clearly defined associated risk factors such as low Apgar scores, congenital heart disease, and sepsis. Intra uterine growth restriction (IUGR) in term neonates also poses a significant risk.[41] This is due to the foetal circulation redirecting blood away from the mesenteric system, towards more vital organs such as the brain, heart and kidneys during development, leading to impaired intestinal development and maturation.[12]

Regarding birth weight, the risk of developing NEC is indirectly proportionate to birth weight, as it rises with decrease in birth weight, with an incidence rise of about 7% between VLBW neonates and extremely low birth weight (ELBW) neonates.[42,43]

It seems obvious therefore that prevention of premature births, and IUGR, should lead to a decrease in the incidence of NEC. Improved antenatal care is the best way of attempting to achieve this goal.

2.4.2 Neonatal hypoxia

The first few days in a neonate's life is critical. Any instability in perfusion to the bowel during this period could increase the risk for development of NEC. Tissue perfusion is dependent on the oxygen delivery to tissue (D02), and the oxygen consumption of that tissue (VO2).[44]

$$DO2 = CaO2 \times CO$$

$$VO2 = CO \times (CaO2 - CvO2)$$

Thus, the most important physiological parameters that determine oxygen delivery and consumption at tissue level are the oxygen content of arterial blood (CaO2), and the cardiac output (CO). Any changes in these two parameters could negatively impact the tissue perfusion to the intestines.[44]

$$CaO2 = (1.34 \times (Hb) \times SpO2) + (0.003 \times PaO2)$$

This equation shows us that the oxygen content of blood is dependent on the patient's arterial oxygen saturation (SpO2), and haemoglobin (Hb) levels (thus oxygen carrying capacity). Any drop in oxygen saturation due to hypoxia would lead to a drop in the oxygen content of the blood. Due to shunting mechanisms redirecting blood to the more vital organs, the tissue perfusion to less vital organs such as the intestines would drop, leading to poor perfusion in the mesenteric beds.[44]

Conditions or interventions that could cause hypoxia in neonates include the following:

- Recurrent apnoea
- Respiratory distress syndrome
- Umbilical artery catheterisation
- Birth asphyxia
- Maternal cocaine use
- Exchange transfusions[12]

Thus, preterm neonates need to be monitored very closely, especially in the first week of life, for any incidents that could lead to hypoxia. Adequate resuscitation at delivery, good communication with the obstetric team, avoidance of umbilical vessel catheterization, and continuous apnoea monitoring are all interventions that can be suggested in this regard.

2.4.3 Formula feeding

As discussed previously in the pathophysiology section (See 2.3.3 Feeding and mechanical injury) timing, volume and type of feeds has been shown to be important variables in the development of NEC. The most important variable that has been proven to be of clinical importance is the difference between exclusive breastfeeding, and formula feeds.[41] Exclusively formula fed neonates have been reported to be three times more likely to develop NEC than their breastfed counterparts.[12]

The important differences between breast and formula feeds implicated in this increased risk are as follows:

- Different probiotic make-up
- Higher protein content in breastmilk
- Higher caloric density in breastmilk
- Higher osmolality in formula milk
- Higher levels of free fatty acids in formula milk
- Lack of maternal protective factors in formula milk[12]

The fact that breastfeeding is proven protective against the development of NEC, especially in preterm neonates, is an easy, inexpensive, practical way of eradicating an important risk factor for NEC, especially in lower income countries, as it is a free resource. The need to promote breastfeeding and assist mothers of preterm neonates in achieving breastfeeding goals cannot be emphasized enough.

2.4.4 Antenatal steroids

Antenatal steroids in the form of dexamethasone or betamethasone, are given to mothers who go into preterm labour between 24 and 34weeks, to assist in foetal lung maturation. If the steroids are given and delivery can be delayed to 24hours after the first dose, deaths from neonatal respiratory distress have been shown to significantly decrease.[45]

Various studies have been done implicating antenatal steroids as both a risk factor and a protective factor in the development of NEC. However, all the studies agree that potential benefit gained from the respiratory effects of antenatal steroids, out way the risk of development of NEC. Thus, the use of antenatal steroids to promote respiratory health should not be discouraged.

2.4.5 Congenital heart disease

As discussed in the section on neonatal hypoxia as a risk factor (2.4.2 Neonatal hypoxia) cardiac output (CO) has an important influence on tissue oxygen perfusion.

$$CO = HR \times SV$$

The cardiac output is directly influenced by heartrate (HR) and stroke-volume (SV).[44] Neonates with congenital heart disease, specifically those with a left-to-right shunt, such as patent ductus arteriosus, have a decreased stroke-volume, and since neonates already have a high baseline heartrate, they have a limited capacity to compensate by increasing their heartrate. Decreased cardiac output will lead to decreased tissue oxygen delivery, and impaired perfusion in the mesenteric bed.[12]

Since congenital heart disease cannot in essence be prevented, the emphasis here should fall on early detection and management of any cardiac conditions in preterm neonates. By detecting conditions early, management can be aimed at improving the stroke-volume and preventing

shunting. All neonates with congenital heart lesions that are significant enough to impair cardiac output, should be managed in centres with specialist paediatric cardiologists.

2.5 Diagnosis

2.5.1 Clinical

Recognition of the clinical signs of NEC is of great importance to any doctor working with neonates, due to the possibility of rapid and fatal progression of this disease. Early signs are very non-specific, and can include any or all the following:

- Apnoea
- Changes in baseline heartrate
- Changes in level of consciousness or irritability
- Temperature instability

Of these early clinical markers, changes in heartrate has been looked at the most in studies. In 2013 Utone et al demonstrated that increased heart rate variability could be identified up to 18hours prior to NEC being diagnosed in neonates in an intensive care unit. By continuous heart rate monitoring, they defined increased variability by increases in decelerations, and decreases in accelerations.[46] More recently, a study conducted at the University of Pretoria in South Africa, demonstrated a persistent increase in heart rate 72hours before neonates with confirmed NEC deteriorated up to the point of needing surgical intervention.[47] Both of these studies indicate the importance of changes in heartrate as an early, and valuable identification tool in neonates who both develop NEC, and subsequently deteriorate.

Signs which implicate the gastro-intestinal tract specifically include:

- Feeding intolerance
- Increased gastric aspirates
- Bilious/bloody vomiting
- Abdominal distention
- Blood per rectum

Subsequently abdominal tenderness, discoloration of the abdominal wall, and a palpable abdominal mass, will indicate progression of the disease process.[17] Recurrent, single observer examination of the abdomen is necessary to pick up any deterioration which might indicate bowel necrosis. It is also important to note that the disease process can progress rapidly, and thus once the diagnosis of NEC has been made, the neonate needs to be placed in a high care unit at least, and clinically reviewed no less frequently than every 6-hours.

An important distinction is also made between the presentation and onset of symptoms in preterm neonates, as opposed to term neonates who develop NEC. Preterm neonates tend to develop NEC after 15 – 20days of life, whereas term neonates develop NEC within the first few days of life.[8]

2.5.2 Radiological

However, to confirm the presence of NEC, radiographic evidence is required. Although clinically suspected, the diagnosis needs to be confirmed by certain signs on plain abdominal X-rays.

- Pneumatosis intestinalis
- Adynamic ileus
- Air in the portal venous system (branching radiolucency extending to within 2cm beneath the liver capsule)
- Pneumoperitoneum
- Ascites

The amount of non-pathological intra-mural gas that can be detected may vary with gestational age. Most term neonates will exhibit some evidence thereof on plain abdominal films, while the presence and possible identification of pneumatosis in preterm neonates may be impaired.[42] The use of serial X-rays can assist if doubt regarding the diagnosis exists.

Another imaging modality that can be utilized in the diagnosis and management of NEC is abdominal ultrasonography. The additional use of colour duplex Doppler imaging can assist in evaluating the thickness of the bowel wall, the echogenicity thereof, and appraising the quality of bowel wall perfusion, as well as identifying fluid collections, ascites, peristalsis, and pneumatosis too small to be visible on plain films.[17] However, ultrasound is highly user dependant, and a qualified Radiologist with experience in neonatal imagining is needed to make the diagnosis using this modality.

In some hospitals, abdominal ultrasonography can be of use to evaluate the presence of necrotic bowel; however, the limitations thereof include inter-observer variability, oversensitivity for detecting abnormalities, and limited availability in all settings.

Imaging modalities that have not proven of practical value in the diagnosis and evaluation of NEC include:[8]

- Contrast screening studies
- Computed Tomography (CT)
- Magnetic Resonance Imaging (MRI)

2.5.3 Laboratory studies

Although very non-specific in making the diagnosis of NEC, biochemical and haematological evaluation can assist in the management of the patient, and identification of the presence of bowel necrosis. Common findings may include:

- Neutropenia or neutrophilia
- Thrombocytopenia
- Metabolic acidosis (persistent)

- Hypo- or hyperglycaemia
- Electrolyte disturbances (particularly hyperkalaemia and hyponatremia)

To assist in confirming the diagnosis and planning the management thereof a standardized scoring system was created. A combination of clinical and radiological evidence makes scoring the severity of the insult possible, by making use of the Modified Bell's Staging (Table 1).[1] Laboratory studies usually reveal a nonspecific inflammatory process. Currently there is no specific test available to confirm NEC or predict the course thereof.

STAGE	CLINICAL FINDING	RADIOGRAPHIC FINDINGS	GASTROINTESTINAL FINDINGS
I: Suspected **A**	Apnoea Bradycardia Temperature instability	Suggestive of ileus only	Increased NG aspirates Occult Blood in stool Mild abdominal distention
B	Same as IA	Same as IA	Same as IA, plus gross blood in stool
II: Definite **A – Mildly ill**	Apnoea Bradycardia Temperature instability	Ileus with dilated loops Focal pneumatosis	Macroscopic blood in stool Severe abdominal distention Absent bowel sounds
B – Moderately ill	Thrombocytopenia Mild metabolic Acidosis	Widespread pneumatosis Ascites Portal Venous Gas	Abdominal wall oedema Tenderness Palpable bowel
III: Advanced **A – Severely ill, bowel intact**	Mixed acidosis Oliguria Hypotension Coagulopathy	Prominent bowel loops Worsening ascites No free air	Worsening wall oedema Erythema Induration
B – Severely ill, bowel perforated	Shock Deteriorating Laboratory values and Vital Signs	Pneumoperitoneum	Perforated bowel

Table 1: Modified Bell's Staging for Necrotising Enterocolitis [1]

2.6 Differential diagnosis

From the previous section, it should be clear to the reader that the diagnosis of NEC is difficult to make due to the non-specific presenting symptoms. Especially in the early stages, a few other disease processes found in neonates could mimic the symptoms of NEC.

- Hirschsprung's disease (with or without Hirschsprung's associated enterocolitis)
- Ileus due to sepsis
- Malrotation with midgut volvulus

- Meconium ileus
- Small bowel atresia (especially Type 0)
- Spontaneous intestinal perforation (SIP)
- Gastric perforation
- Maternal ingested blood
- Food protein-induced allergies [48]

All these conditions need to be investigated and actively excluded. Malrotation with midgut volvulus is a surgical emergency that can have devastating consequences if the diagnosis is missed.[1] Spontaneous intestinal perforation (SIP), also known as focal intestinal perforation, can have a very similar clinical presentation as NEC Grade IIIB, and essentially the management always requires surgical intervention, whether by the insertion of peritoneal drains, or laparotomy and resection of the perforated segment. The difference between SIP and NEC only becomes evident during surgery, and at histological review of the resected bowel.[49]

2.7 Prevention

Previously the focus of neonatologists was solely on the management of NEC once it had already developed. This unfortunately did not lead to any significant improvement in the outcomes or long-term complications. Currently the focus of research has moved more towards identifying possible causative factors and preventing the development of NEC in susceptible neonates. Thus far, the main focus in prevention of NEC has been adjustments in feeding strategies and modifying the gastro-intestinal flora.

2.7.1 Feeding Practices

Where feeds are concerned, the time of initiation of feeds, type of feeds (breastmilk versus formula feeds), fortification of feeds, and rate of escalation of feeds, are important variables. Initiating early feeds in preterm neonates has been thought to increase the risk for developing NEC, although a systemic review by Kennedy and Tyson in 2000 concluded that the available studies had a too small sample size to adequately back this finding.[50] Neonates who are breastfed have been found to have a lower risk of developing NEC than those who receive formula feeds, independent of the age at initiation of feeds.[51,52] Although some studies have suggested that a higher rate of advancement of feeds once initiated carries a higher risk for developing NEC,[53] the results from these studies are still being scrutinized.

Regarding donor breastmilk, there is some data to suggest its use rather than formula milk. However, the breastmilk of mothers of premature neonates have proven to have lower levels of interleukin 10 (IL-10) than the breastmilk of mothers with term babies. IL-10 assists in preventing NEC by downregulation of inflammatory processes and prevention of over-production of pro-inflammatory cytokines. Donor breastmilk might not contain the same anti-inflammatory components necessary for VLBW neonates, and the pasteurization process can affect the make-up significantly.[54] Current feeding strategies at the NICU's in PTH and UAH include delaying feeds in preterm neonates, slower rate of feed increase in preterm neonates, and advocating for breastmilk feeds rather than formula feeds whenever possible.

2.7.2 Probiotics and prophylactic antibiotics

In addition to changes in feeding strategies, the use of enteral antibiotics and probiotics has been suggested to protect against NEC,[42] but this is still controversial and is not currently being utilized at any of our local units. Intestinal dysbiosis and overgrowth of pathogens in the neonatal intestinal tract is a proven entity. The role of probiotics would be to restore normal commensal bacteria in an attempt to prevent the development of NEC in at risk neonates.

The biggest risk of giving prophylactic probiotics to neonates is the development of probiotic induced sepsis due to the immature immune system of premature neonates.[42] In a meta-analysis done evaluating 11 studies done to examine the role of probiotics in preventing NEC in VLBW neonates, only 1 out of 25 patients benefited from the use of probiotics.[55]

The first multimodal protocol introduced for prevention of NEC was done after a study done at the Graz University in 2006. The Graz protocol consisted of early enteral feeds with exclusive breastmilk, as well as an enteral antibiotic (Gentamycin), an enteral probiotic (*Lactobacillus Rhamnosus*), and an enteral anti-fungal agent (Nystatin). Feeds are also increased slowly, starting with 1ml/kg every 3hours, and increased by only 1ml/kg per feed every day. When this protocol was introduced within the first 24hours of life in a group of premature neonates, the incidence of NEC was only 0,7%, with a significant difference from a control group of premature neonates who did not receive the protocol.[56]

2.7.3 Other

Some other substances have been suggested to prevent the development of NEC, but most of the are still under investigation, and not widely available.

Epidermal growth factor (EFG) is a substance known to be of importance in maturation of the foetal gut. Neonates who are born prematurely have been proven to have significantly lower levels of salivary EGF than term neonates, and neonates who developed NEC showed a similar paucity in EGF. This could indicate that administration of external EGF could have a protective role in the neonatal gut. Studies in rats have shown a reduction in the development on NEC, normalization of intestinal villi structure, as well as a decrease in the production of pro-inflammatory cytokines, and an upregulation of anti-inflammatory cytokines, in premature rats who were given oral EGF. However, as no EGF that is suitable for administration to premature neonates is available yet, there is still a way to go with this hypothesis.[54]

Another protective intervention that has enjoyed some attention in the medical community is the use of oral immunoglobulins (IgG and IgA). Immunoglobulins have a protective effect in the premature neonate's gastrointestinal system, but they have lower levels of circulating immunoglobulins than their term counterparts. Premature neonates who are breastfed receive some specific immunoglobulins from their mothers, but the ones who are formula fed do not. Two important studies done in 1988 and 1991 showed potential for the use of oral immunoglobins to prevent NEC in premature neonates. Both of these studies reported a reduced incidence of NEC in low birth weight neonates for whom breast milk from their mothers was not available, when oral immunoglobulins were administered.[57,58]

After these two ground-breaking studies, two similar studies were done to try and recreate these findings. Richter et al in 1998 did a similar study where they showed that ELBW neonates were

not protected from NEC by oral immunoglobulins, and they could not recommend it as a prophylactic intervention.[59] In 1994 Fast et al compared the use of oral immunoglobulins to the use of oral gentamycin as prevention for the development of NEC, and found oral gentamycin superior in its prophylactic efficacy.[60] Most recently a Cochrane review concluded that based on the current available evidence in the literature, the use of oral immunoglobulins for prevention of NEC is not recommended.[61]

Some other methods for prevention that have been suggested, but are unproven or still under investigation, includes glucocorticoids, arginine, glutamine, and inflammatory cytokine receptor blockers.[54,62]

2.8 Management

The management of NEC depends on the clinical condition of the neonate, with surgical intervention only being utilized once necrosis or subsequent perforation of the bowel is present. Initial treatment is aimed at general patient support, as well as finding and reversing the causative factor, and managing the bacterial component with antibiotics. The most important part of the management of neonates with NEC is deciding who needs surgical intervention, and the timing thereof. Once a patient has been diagnosed with NEC, they need to be very closely monitored to pick up any detrimental changes timeously, and act upon them.[8]

2.8.1 Medical management

A septic work-up is done on all neonates showing signs of NEC, after which empirical antibiotic therapy will be commenced. All enteral feeds are discontinued to minimize the risk of further damage to the diseased intestine, and a nasogastric tube (NGT) is inserted to decompress the stomach and proximal bowel. Parenteral feeds to optimize nutrition, and intravenous fluid therapy is also started.[23]

All neonates with clinical signs and symptoms suggestive of NEC need to be closely monitored with serial blood-gas analysis, abdominal examination, and abdominal X-rays at least every 6 – 8hours initially to quickly pick up any deterioration in the early stages of the disease.[63]

Grade I and II disease can mostly be managed conservatively, with clinical improvement expected within 48 - 72 hours, after which feeds can be restarted and increased as tolerated.[8] Lack of clinical improvement, or deterioration in clinical state should prompt involvement of the surgical team, and possible re-evaluation of the diagnosis.

In a recent study published in March 2020, a modified version of the Graz protocol (See section 2.7.2) was given to patients with early NEC. An enteral antibiotic (Gentamycin), enteral probiotic (synbiotic LactoG), and enteral anti-fungal agent (Nystatin) was therapeutically given to a group of neonates with confirmed early NEC. This regimen significantly decreased the NEC associated morbidity and mortality in the intervention group compared to the control group.[34] Although this study was a first in the field, and the results can make a big difference in the future management of NEC, further studies are needed to verify these findings.

2.8.2 Surgical management

Some controversy still exists regarding the exact timing of surgical intervention, but the most commonly utilized clinical parameters include bowel perforation (distinguished by

pneumoperitoneum on abdominal X-rays, or the finding of bowel content on paracentesis).[1] Absolute indications for surgery include:

- Palpable abdominal mass (suggestive of necrotic bowel with sealed off perforation)
- Clinical deterioration despite maximum medical intervention
- Evidence of intestinal obstruction on plain abdominal film
- Evidence of adynamic segment of bowel on plan abdominal film (suggestive of necrotic bowel)
- Worsening biochemical picture

Relative indications for surgery include:

- Increased abdominal tenderness, distention, or discolouration
- Portal vein gas
- Pneumoperitoneum
- Positive paracentesis
- Severe thrombocytopenia.

Patient with severe NEC Grade IIIB may be extremely ill and may require aggressive resuscitation before and during surgery. Blood products, inotropic support, and ventilatory support need to be available immediately.

Surgical intervention ranges from primary peritoneal drainage, to laparotomy and resection of necrotic bowel. Peritoneal drainage can be used in neonates who have developed respiratory compromise due to extensive pneumoperitoneum or ascites, in an attempt to stabilize them for transfer to theatre. It has also proven valuable as a primary intervention for Grade IIIB NEC in some units, although there are studies that have disproven its use as a sole therapeutic intervention.[8,64]

The goal or surgery is to remove the necrotic bowel, whilst aiming to leave enough viable small bowel to prevent the development of short bowel syndrome. Depending on the clinical condition of the patient during surgery, the surgeon might decide to resect the necrotic bowel and do a primary anastomosis in a very stable patient with normal clinical and biochemical parameters, resect the necrotic bowel and bring out stomas if the bowel is in poor condition or the patient's condition is not favourable, or do a clip-and-drop surgery with the aim of definitive surgery later when the patient is more stable.[8,19,65] All options have been reported and are acceptable, and it remains at the discretion of the surgeon to decide what would be the best option for their patient.

Laparoscopy may be useful in confirming the presence of necrotic bowel or sealed of perforations, but once identified the recommendation is that the surgery be converted to an open laparotomy.[66]

2.9 Transfusion Associated NEC (TANEC)

2.9.1 Definition

Transfusion Associated Necrotising Enterocolitis (TANEC, referred to as TRANEC by some authors) refers to NEC that develops in neonates within 48-hours after receiving an RBC transfusion.[67] A study in Canada in 2013, by Stritzke et al, showed that neonates with a lower mean gestational age and lower mean birth weight were at higher risk for developing NEC within two days after receiving an RBC transfusion. However, they did not identify any difference in morbidity or mortality between the patients who had developed TANEC, and the neonates who had developed NEC without receiving an RBC transfusion.[68]

The association between transfusion of blood products and the development of NEC in preterm neonates was first described in 1987 by McGrady et al,[69] although very little further research was conducted into this phenomenon until the last decade. This has led to larger neonatal centres reviewing their transfusion strategies, as well as adopting protocols where at risk neonates do not receive enteral feeds immediately before and after a transfusion of blood products. There is however still wide spread controversy regarding this topic, with some studies showing no significant correlation between transfusion of RBCs and the development of NEC.[70]

2.9.2 Pathophysiology

TANEC, also called transfusion related acute gut injury (TRAGI) in some units, is thought to be an adverse reaction to blood transfusions, similar to the pulmonary counterpart found in adults, namely "transfusion related acute lung injury" (TRALI).[71] This pathological process is hypothesized to be due to a multifactorial insult on the intestinal barrier. Firstly, at cellular level, there is thought to be an immunological response to Human Leucocyte Antigen (HLA), and other antigens present in the transfused blood. This leads to a cascade of endothelial cell activation.[3] This immune reaction, in combination with the increased viscosity of the blood products, is thought to lead to a decrease in mesenteric blood flow directly after transfusions. Along with this, enteral feeds in the presence of decreased mesenteric blood flow can lead to the development of NEC.[6] The last factor influencing the perfusion state of the bowel after a blood transfusion, can be the age of the donor blood, leading to a decrease in capacity to deliver oxygen to tissues, and subsequently to vasoconstriction and ischemia.[72]

2.9.3 Transfusion guidelines

The neonatal population receive some of the highest amounts of blood transfusions in hospitals. However, very few guidelines exist regarding the threshold for transfusions in neonatal units. Even where guidelines do exist, it is seldom adhered to. This is mostly due to the previous school of thought being that to optimize the oxygen delivery to tissues, the haemoglobin delivery system should be optimized by transfusing packed cells. However, recent studies have shown that unnecessary transfusions of RBCs have more detrimental effects in the neonatal population than previously thought. Also that oxygen delivery to peripheral tissues may be impaired at too high an artificial haematocrit.[73]

Three studies done between 2006 and 2011 demonstrated that up to a third of neonates who had developed NEC, had received RBC transfusions within 48-hours prior to the development of the NEC.[74,75,76] However, some controversy still exist regarding whether it is the RBC transfusion

that predisposes neonates to the development of NEC, or the pre-existing severe anaemia. In 2016 Patel et al conducted a observational cohort study, evaluating the these two entities, and identified that the severe anaemia, rather than the RBC transfusion was the bigger risk factor for the development of NEC.[77]

This possible existence of TANEC has led to more scrutiny in the practice of RBC transfusions. In 2009 Valieva et al published a study evaluating the previous University of Washington Neonatal Intensive Care Unit 2006 Transfusion Guidelines,[78] and found that no clinical benefit to giving RBC transfusions above the limits of their new clinical guidelines (Table 2) could be identified. They found that more neonates needed respiratory support after transfusion and identified a positive link between RBC transfusions and Bronchopulmonary Dysplasia in neonates. They also identified a possible association between RBC transfusions and the development of NEC, and as a result of their study, the guidelines were amended to allow for lower haematocrit levels in neonates, as well as a more clinical approach to deciding whether or not to transfuse a patient.[73]

The most commonly used guideline for RBC transfusion in neonates, are as set out in Table 2.[73]

Indications for Transfusion of RBC's in Neonates	
HCT	**AGE and CLINICAL CONDITION**
<35%	≤1week of Life **AND** Unstable*
<28%	≤1week of Life **OR** Unstable*
<20%	>1week of Life **AND** Unstable*
*Unstable clinical condition is defined as a state of poor oxygen delivery, e.g. Respiratory distress/apnoea, Cardiovascular deterioration etc.	

Table 2 : Indications for RBC transfusions in neonates [73]

2.9.4 Feeding during transfusion

As mentioned before, the impact of enteral feeds on mesenteric blood flow and oxygen delivery during RBC transfusions, is of great importance. A study conducted by Krimmel et al in 2009 found that there was decreased flow in the Superior Mesenteric Artery (SMA) postprandially following an RBC transfusion, suggesting an increased risk for intestinal ischaemia.[79] Wan-Huen et al did a similar study in 2013 which found that neonates who were fed within two days before receiving an RBC transfusion were eight times more likely to develop TANEC.[72] By making use of near infrared spectroscopy in measuring the mesenteric regional oxygen saturation in neonates receiving transfusions, Marin et al showed a decrease in mesenteric oxygenation in patients receiving concurrent feeds, as opposed to an increase in patients in whom enteral feeds were halted during the transfusion. They also found that more premature neonates were increasingly vulnerable to changes in mesenteric blood flow after RBC transfusions, and subsequent mesenteric ischaemia.[6] This study created a physiological base for the findings in the previous studies.

In contrast, the FEEDUR RCT trial conducted in Australia between 2016 and 2017, proved no difference in splanchnic oxygen delivery in infants that received enteral feeds during RBC transfusions. They measured the splanchnic-cerebral oxygen ration (SCOR) and mean splanchnic fractional oxygen extraction (FOE), before during and after RBC transfusion in 60 neonates. Also

of note, none of the neonates included in this study developed NEC, but the incidence of NEC in VLBW neonates in the hospital in question is significantly lower that the international standard, and this needs to be taken into account, along with the small sample size of the study.[80]

The only definite guideline that exists for keeping neonates NPO peri-transfusion, is the Duke Intensive Care Nursery protocol first implemented there in February of 2009. This states that all enteral feeds and fluids should be withheld for four hours before, during, and after the RBC transfusion. A study done by DeRienzo et al in 2014 monitoring the effect of feeding practices in the development of TANEC, made use of this protocol to evaluate their findings.[81] They saw a decrease in the incidence of NEC in their units following improved transfusion practices, although a clear link between the change in feeding protocol and the development of TANEC could not be proven due limitations in their study.

Lastly of note is the different feeds that can be administered to VLBW neonates. The protective effects of breast milk feeds in VLBW neonates has been well established, and its importance in the prevention of NEC cannot be overstated. In a Cochrane review in 2008, eight studies that evaluate the effect of breastmilk on various areas of neonatal health and development were compared. They found that formula feeds had a 2.5% relative higher risk for developing NEC, compared to donor milk.[82] There are currently no studies comparing breastmilk versus donor milk in the development of TANEC.

2.9.5 Exchange transfusion

Indications for exchange transfusions in neonates include:[83,4,84]

- Severe hyperbilirubinemia due to haemolysis:
    - Maternal blood incompatibility
    - Enzymopathies
    - Membranopathies
- Severe Hyperkalaemia
- Gestational alloimmune liver disease (GALD)
- Severe sepsis
- Disseminated intravascular coagulation (DIC)

The guidelines regarding exchange transfusion in neonates are better researched and established. Exchange transfusions have been identified as an independent risk factor for the development of NEC.[26]

2.10 Prognosis

Since the first description of NEC in the 1980's there has been extensive research done into the cause and treatment thereof. Significant advances in neonatal care in the past two decades have led to improved survival for premature infants, but the mortality rate for neonates with NEC have stayed the same at between 15% and 30%.[14,15,85] The mortality rate for neonates with NEC who require surgical intervention is much higher (up to 50%), but has decreased slightly over the past

few decades due to improved diagnostic, anaesthetic, and surgical skills. This can be attributed to the fact that once they reach the disease severity where they require surgical intervention, neonates tend to be clinically unstable, and have developed failure of other organ systems. The risk that the high mortality rate is due to late identification of the need for surgery needs to be kept in mind though, to help prevent this complication.[8,85]

In 2006, Kessler et al developed a presurgical mortality scoring system for neonates with NEC.

	0	1	2
Gestational age	>32 weeks	32 – 30 weeks	<30 weeks
Bell's Stage	I	II	III
Platelets (10^9/L)	>200	200 – 150	<150
Lactate (mg/dL)	<2.5	2.5 – 3.3	>3.3

Table 3: Presurgical NEC Mortality Score[86]

A score of between 1 and 3 had a 0% mortality, a score of between 4 and 6 had a 20 – 30% mortality rate, a score of 7 had a 60% mortality rate, and a score of 8 carried a 100% mortality rate. The predictive sensitivity of this scoring system was 0.72, with a specificity of 0.72.[86]

2.11 Complications and long-term sequelae

Other than the high mortality rate associated with NEC, neonates who develop and are successfully treated for NEC also face a barrage of short- and long-term complications, including but not limited to the following:

- Recurrence of NEC (5% risk)[8]
- Sepsis
- DIC
- Shock
- Multi-organ dysfunction and failure
- Intestinal obstruction due to strictures
- Short bowel syndrome
- Entero-cutaneous fistulae
- Entero-colonic fistulae
- Impaired neurological outcome [8,2]

Regarding short bowel syndrome: not only neonates who were managed surgically and had bowel resections are at risk, but also the ones who were managed conservatively. This is due to impaired absorptive capacity of the diseased bowel. Up to 42% of neonates who required surgical intervention for NEC will subsequently develop short bowel syndrome.[8]

Premature neonates are at increased risk for neurodevelopmental delays due to the interruption of their intra-uterine development. NEC and sepsis added on to this already significant risk, can lead to devastating long-term neurological complications. Reese et al. reported that up to 45% of neonates who survive NEC have a poor neurodevelopmental prognosis, with the risk being even higher for neonates who received surgical intervention. These included neurodevelopmental problems such as deafness, cerebral palsy, visual impairment, mental developmental impairment, and psychomotor impairment.[87]

2.12 Conclusion

There is no question about the importance of further insight and research into NEC being of vital importance to the preterm neonatal population. NEC is a common, life-threatening disease with an increased incidence seen due to improvements in neonatal care. The burden NEC places on health care systems, hospitals, neonatal personal, as well as society due to the long-term complications, cannot be ignored.

RBC transfusions are not without consequences, despite their often lifesaving impact. It is important that the decision to give a VLBW neonate an RBC transfusion, always take all the potential risks and benefits into account. To aid in this decision making, protocols and guidelines need to be in place.

Currently very little is known regarding the exact aetiology of this devastating condition, and our focus has only recently started shifting away from management of NEC once it has developed to finding causes and risk factors and attempting to prevent the condition. Since the mindset is changing, the focus needs to shift to finding possible causes and doing adequate research to enable evidence based clinical practice.

Chapter 3: Problem Statement

3.1 Problem statement

Is there an association between RBC transfusions in VLBW neonates, and the development of NEC? If so, is there an association with the severity of the ensuing NEC?

3.2 Aims and objectives

The aim of this study is to evaluate the RBC transfusion practices in VLBW neonates in two Academic Neonatal Units in Bloemfontein, Free State, in association with the development of TANEC.

3.2.1 Primary objectives

The aim of this study is to evaluate the RBC transfusion practices in VLBW neonates in two Academic Neonatal Units in Bloemfontein, Free State, in correlation with International Guidelines by:

- Comparing and describing the RBC transfusion practices between the two hospitals
- Recording any changes in RBC transfusion practices over a 5-year period
- Evaluating possible differences in outcomes, specifically pertaining to NEC

3.2.2 Secondary objectives

Additionally, the aim is to gain further insight into the impact of RBC transfusion practices in VLBW neonates by evaluating:

- The haematological indications for RBC transfusions
- The clinical state of neonates during RBC transfusions
- Whether or not they were receiving enteral feeds during the RBC transfusion
- The possible association between RBC transfusions, the timing of enteral feed in relation to the RBC transfusions, and the development of NEC, including its severity

Evaluation of the RBC transfusion practices in association with the development of NEC will aid us in determining whether an association exists between RBC transfusions in neonates, the time frame of enteral feeds in relation to the transfusions, and the development of NEC, as well as the severity of the NEC developed. By evaluating these practices over a 5-year period and being able to identify possible shortcomings in the RBC transfusion protocols, we hope to significantly improve our clinical practice.

Chapter 4: Research Design

3.1 Introduction

This chapter serves as an explanation of the method of data collection by the principle investigator, the data form used to capture the data, and the statistical methods used to interpret the data.

3.2 Pilot Study

A brief pilot study was conducted to evaluate the feasibility of data collection, and the adequacy of the data form used to document the study parameters. This pilot study included 20 patients and showed us that the initial plan to make use of the statistics of the Department of Paediatrics to capture patients, would not be possible due gaps in their statistics. A new method to locate VLBW neonates who were treated at UAH and PTH during our study period was then devised, by using the online statistics captured by the online Meditech© system. This new method proved accurate, although significantly more time consuming, and the study could move forward, albeit with a new adjusted timeline. Since no changes were made to the study protocol or data collection sheet after completion of the pilot study, the 20 patients whose data was collected in the pilot study, were also included in the final study data.

3.3 Basic data collection

The study is an analytical retrospective review.

This study was conducted using data collected from the Neonatal Units of Universitas Academic Hospital, and Pelonomi Tertiary Hospital Bloemfontein, South Africa.

Patient inclusion criteria:

> All Very Low Birth Weight (1000g – 1499g) neonates born in the Pelonomi Tertiary Hospital, and Universitas Academic Hospital, and/or admitted to the respective neonatal units in a 5-year period. The time frame for data collected was between January 2012 and December 2016.

Patient exclusion criteria:

- Neonates born in other hospitals, and subsequently admitted after 48-hours of life
- Any other significant congenital abnormalities
- Neonates who demised within 72 hours of life

Data was collected by obtaining the Paediatric Departmental admission statistics from Meditech©. All VLBW neonates were identified. The electronic notes of all the neonates were reviewed, and by correlating these with the NHLS-Labtak results, RBC transfusions were

identified. The date of the RBC transfusion, as well as the pre-transfusion and post-transfusion haematocrit, and the pre-transfusion clinical condition of the patient was recorded.

Furthermore, patients within the VLBW group who developed NEC were identified. It was recorded whether they developed the NEC before an RBC transfusion, or within 48-hours following one.

Principle data collected consisted of the following parameters:

- Date of birth
- Birth weight
- Gestational age
- RBC transfusion related data
    - Date of transfusion
    - Pre-transfusion haematocrit
    - Post-transfusion haematocrit
    - NPO during transfusion (Yes / No)
    - Clinical condition during transfusion
        - Stable
        - Sepsis
        - Respiratory Distress
        - Tachycardia
        - Hypovolemic Shock
        - Apnoea
        - Exchange transfusion
        - Pulmonary haemorrhage
        - Haemolysis
        - Unstable (Not otherwise specified)
    - Ventilatory support during transfusion
        - Room air
        - IPPV
        - NCPAP
        - NHFO
        - HFOV

- NEC related data
    - NEC developed (No / Within 48-hours after RBC transfusion / Before transfusion)
    - Severity of NEC (Modified Bell's Staging)

3.3 Statistical analysis

The data for PTH and UAH patients was captured and analysed separately, to enable a comparison between the two hospitals. The data analysis was done by the Department of Biostatistics at the University of the Free State. Results were summarised by frequencies and percentages (categorical variables) and means, standard deviations or percentiles (numerical variables). Associations were assessed by relative risks with 95% confidence intervals, and appropriate hypothesis testing.

When the parameter data referred to an average value, the Welch t-test was used to determine whether the difference in values between the two groups were statistically significant, using a 0,05 significance level (p-value).

When the parameter data referred to an occurrence level a 2x2 contingency table was drawn up, and the Fisher exact test was used to determine whether the difference in occurrence levels between the two groups were statistically significant, using a 0,05 significance level.

3.4 Ethics

Approval to conduct the study was obtained from the Health Sciences Research Ethics Committee of the University of the Free State, as well as the Free State Department of Health. Patient confidentiality was always respected by assigning patients individual study numbers on data sheets and keeping data on a secure password protected computer. All data will be kept for 15years after conclusion of the study.

HSREC Approval Number: UFS-HSD2017/0616

FSDoH Approval Number: FS_201805_003

Chapter 5: Results

5.1 Introduction

This chapter will discuss the following results obtained from this study.

- Epidemiological data
- RBC transfusion results
- NEC results

5.2 Epidemiological data

From 1 January 2012 till 31 December 2016, 1585 VLBW neonates were admitted and treated in the neonatal units at PTH and UAH. Of these, 1044 (66%) were admitted and treated at PTH, and 541 (34%) at UAH. Over the 5-year study period the number of VLBW neonates treated at each of these hospitals remained stable with no major fluctuations in number. Thus, the amount of VLBW neonates treated at these two units have remained more or less the same throughout the study period.

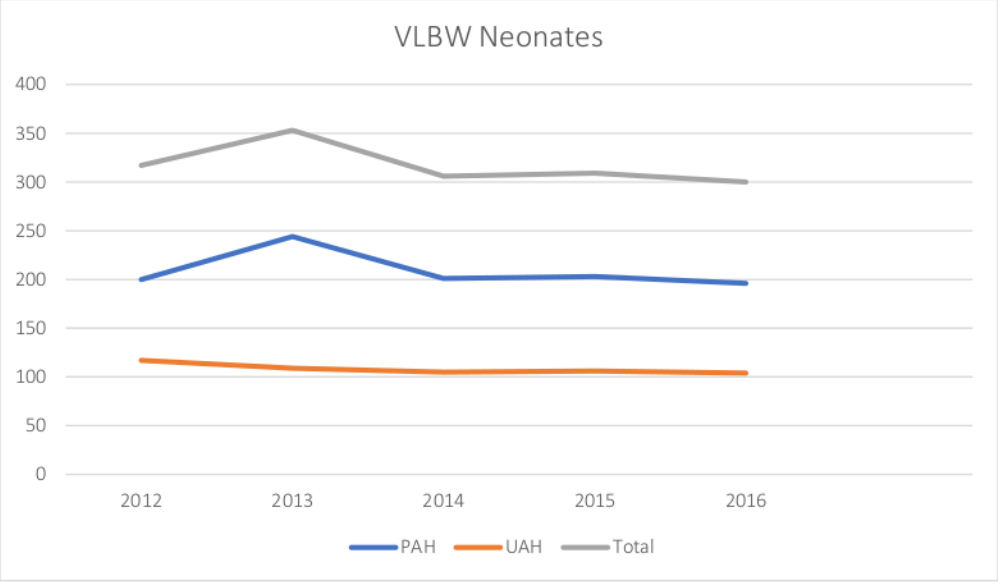

Figure 1: Number of Very Low Birth Weight neonates treated in Pelonomi Tertiary Hospital and Universitas Academic Hospital (2012 – 2016)

Before exclusion of the VLBW neonates who demised within 72-hours of life, together with those who suffered from other significant congenital abnormalities, a profile of the congenital abnormalities found in the VLBW neonates was determined.

At PTH, 63 (6%) of the neonates were excluded because they had demised within 72-hours of life, 18 (1,8%) were excluded due to insufficient data in their files, and 13 (1,2%) were excluded due to other significant congenital abnormalities. The data of 950 (91%) of the 1044 neonates treated at PTH (60% of the total number of VLBW neonates evaluated) were included in the study. The median birth weight of these patients was 1300g, with a median gestational age of 30 weeks.

At UAH, 35 (6,5%) of the neonates were excluded because they had demised within 72-hours of life, 9 (1,7%) were excluded due to insufficient data in their files, and 21 (3,9%) were excluded due to other significant congenital abnormalities. The data of 476 (88%) of the 541 neonates treated at UAH (30% of the total number of VLBW neonates evaluated) were included in the study. The median birth weight of these patients was 1200g, with a median gestational age of 29 weeks.

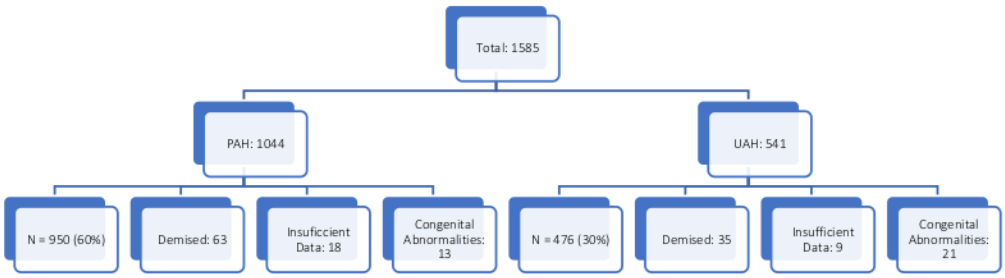

Figure 2: Profile of Very Low Birth Weight neonates excluded from the study (2012 – 2016)

The congenital abnormalities found in VLBW neonates excluded from the study, were as follows:

Congenital Abnormality	N	Prevalence	Percentage of Congenital Abnormalities
Duodenal atresia	7	4,4: 1000	21%
Oesophageal atresia (±Tracheo-oesophageal fistula)	4	2,5: 1000	12%
Malrotation	3	1,9: 1000	9%
Myelomeningocele	3	1,9: 1000	9%
Trisomy 21 (Down syndrome)	3	1,9: 1000	9%
Hirschsprung's disease	2	1,3: 1000	6%
Anorectal malformations	2	1,3: 1000	6%
Gastroschisis	2	1,3: 1000	6%
Trisomy 18 (Edward syndrome)	2	1,3: 1000	6%
Small bowel atresia	1	0,6: 1000	3%
Omphalocele	1	0,6: 1000	3%
Meconium ileus	1	0,6: 1000	3%
Osteogenesis imperfecta	1	0,6: 1000	3%
Noonan syndrome	1	0,6: 1000	3%
Turner syndrome	1	0,6: 1000	3%

Table 4: Profile of congenital abnormalities in Very Low Birth Weight neonates in Bloemfontein Academic Hospitals (2012 - 2016)

Since all the patients with congenital abnormalities are transferred from PTH to UAH there was no distinction made between these two hospitals. The above numbers can therefore be interpreted as the prevalence (2,1%) of significant congenital abnormalities, as well as the prevalence of the respective congenital abnormalities, in VLBW neonates in the Bloemfontein Academic hospitals between 1 January 2012 and 31 December 2016, as depicted by the below graph.

Of the 1585 VLBW neonates treated at PTH and UAH between 2012 and 2016, 34 (2,1%) had congenital abnormalities. The most common congenital abnormality seen in the excluded VLBW neonates in Bloemfontein Academic hospitals was duodenal Atresia (0,44%), followed by oesophageal atresia (0,25%).

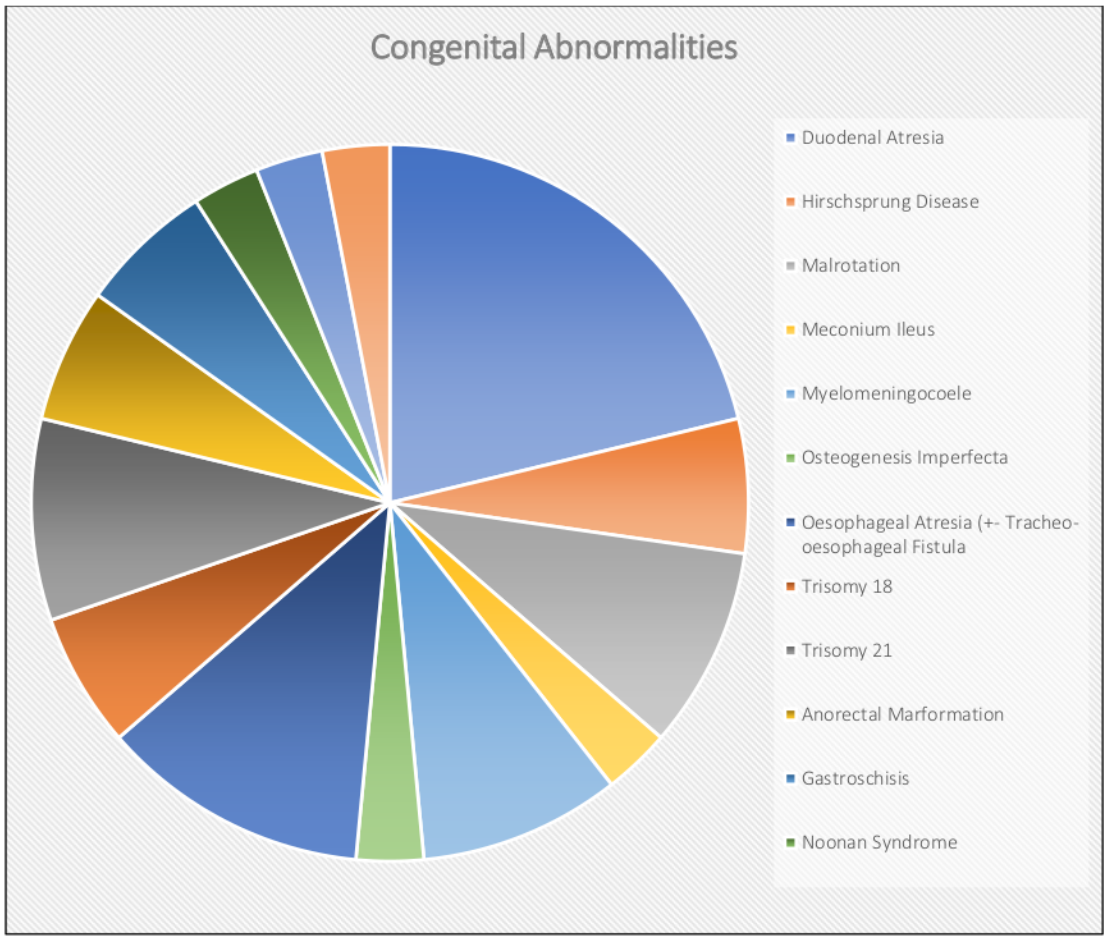

Figure 3: Distribution of congenital abnormalities in Very Low Birth Weight neonates excluded from the study. (2012 – 2016)

5.3 RBC transfusion results

5.3.1 Pelonomi Tertiary Hospital

The median birth weight of the 950 VLBW neonates whose data was evaluated at PTH, was 1300g, with a median gestational age of 30 weeks. Of these, 340 (36%) had RBC transfusions. The median birthweight for the latter was 1220g, with the median gestational age also 30 weeks. This indicates that the VLBW neonates who had received RBC transfusions were representative of the average VLBW neonate treated at PTH (p-value 0,01).

There was a marked change in RBC transfusion practices the 5-year study period, with a decline in the number of VLBW neonates transfused at PTH between 2012 – 2016. In 2012, 114 (57%) of the 200 VLBW neonates who were treated, received RBC transfusions, while in 2016 only 37 (19%) of the neonates were transfused (p-value 0,0005).

The median pre-transfusion haematocrit was 0,33 L/L, with the median post-transfusion haematocrit 0,40 L/L. The neonates who were kept NPO at the time of, and for at least 120minutes after the transfusion, was 172 (50,6%).

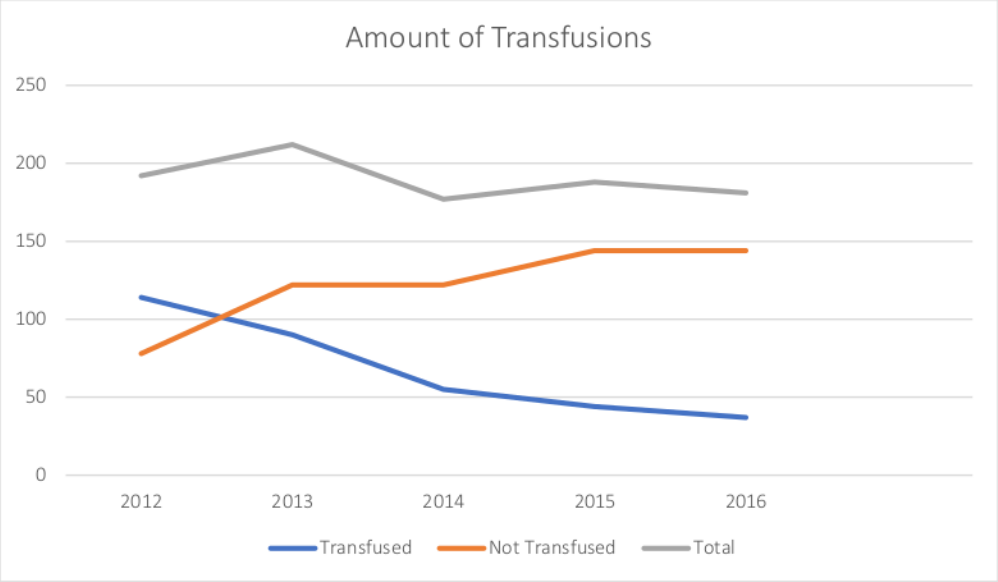

Figure 4: Amount of RBC transfusions in Very Low Birth Weight neonates at Pelonomi Tertiary Hospital (2012 - 2016)

The total number of VLBW neonates who received RBC transfusions at PTH slowly declined from 2012 to 2016, with a relative rise in the number of neonates kept NPO during transfusions. A marked change in the RBC transfusion practices was notes as in 2012, 45 (40%) of the 114 neonates who received RBC transfusions were kept NPO, while in 2016, 23 (62%) of the 37 neonates who received transfusions were kept NPO. This can be correlated with the publication of new transfusion guidelines when TANEC became a more recognisable entity in the medical field.

The total number of VLBW neonates who received RBC transfusions at PTH continued to drop from 114 (57%) in 2012, to 37 (18,8%) in 2016. A percentage rise of 12% of neonates kept NPO during transfusions was demonstrated over the same period. This change in the number of patients kept NPO during RBC transfusion is statistically significant (p-value 0,03).

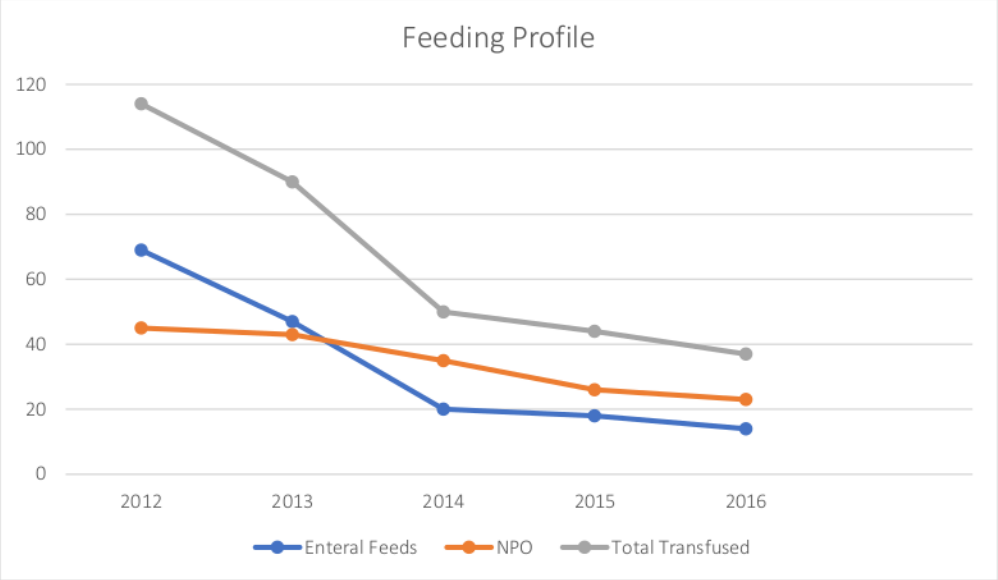

Figure 5: Feeding profile of Very Low Birth Weight neonates transfused at Pelonomi Tertiary Hospital (2012 - 2016)

The clinical conditions of neonates receiving RBC transfusions were the following:

Clinical condition	Number	Percentage
Stable (no clinical abnormality)	204	60%
Septic / Septic shock	40	11,8%
Respiratory distress	23	6,7%
Tachycardia	23	6,7%
Hypovolemic shock	23	6,7%
Apnoea	10	2,9%
Exchange transfusion	8	2,4%
Pulmonary haemorrhage	4	1,2%
Haemolysis	2	0,6%
Unstable (Specific clinical signs not specified)	2	0,6%
Tachypnoea	1	0,3%

Table 5: Clinical condition of Very Low Birth Weight neonates at Pelonomi Tertiary Hospital during RBC transfusion (2012 - 2016)

The vast majority (60%) of the transfused neonates was recorded as clinically stable and had no clinical indication for the RBC transfusion. When correlating this with the average pre-transfusion haematocrit (0,33L/L), neonates at PTH were transfused with RBCs more often than was clinically indicated. The guidelines as discussed in Chapter 2 (See 2.9.3 Transfusion guidelines) indicate that patients with a haematocrit between 0,28L/L and 0,35L/L should only be transfused of they are clinically unstable.[35]

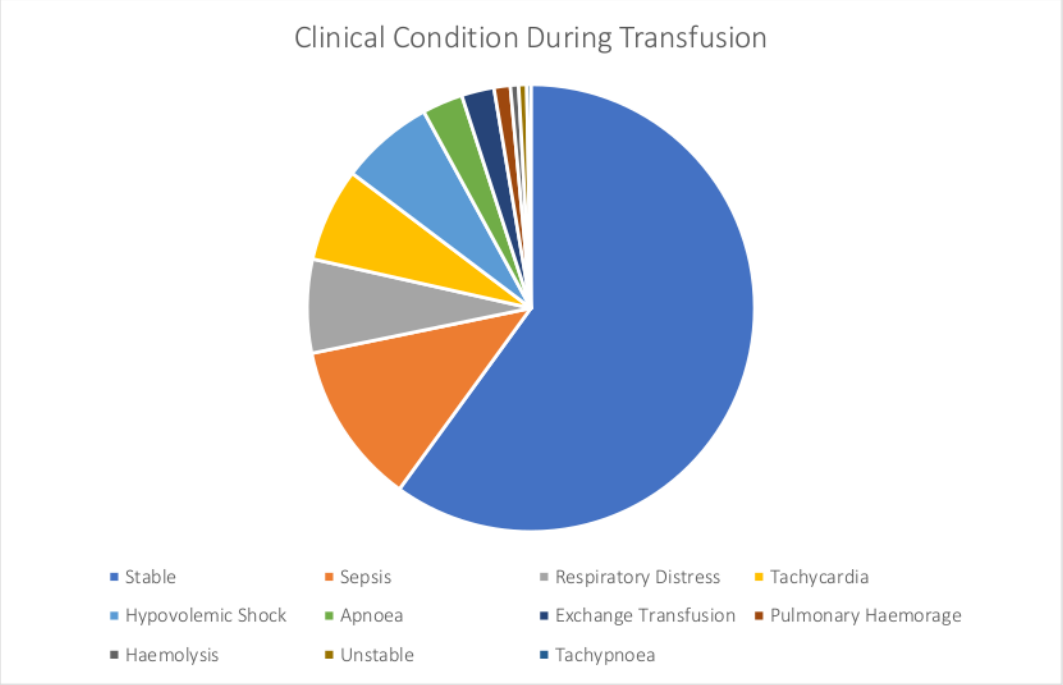

Figure 6: Distribution of clinical conditions of Very Low Birth Weight neonates at Pelonomi Tertiary Hospital during RBC transfusion (2012 – 2016)

The ventilatory support of the neonates who received RBC transfusions was also evaluated, and recorded as follows:

Ventilatory Support	Number	Percentage
High Frequency Oscillatory Ventilation	0 (Not available at PTH at time of study)	0%
Intermittent Positive Pressure Ventilation	30	8,9%
Nasal Continuous Positive Airway Pressure	70	20,6%
Nasal High Flow Oxygen	97	28,5%
Room Air	143	42%

Table 6: Ventilatory support in Very Low Birth Weight neonates at Pelonomi Tertiary Hospital during RBC transfusion (2012 - 2016)

The majority of transfused VLBW neonates (42%) were recorded to have had no respiratory problems and were stable on room-air. This is another pointer that RBC transfusion was not clinically indicated in most of the neonates.

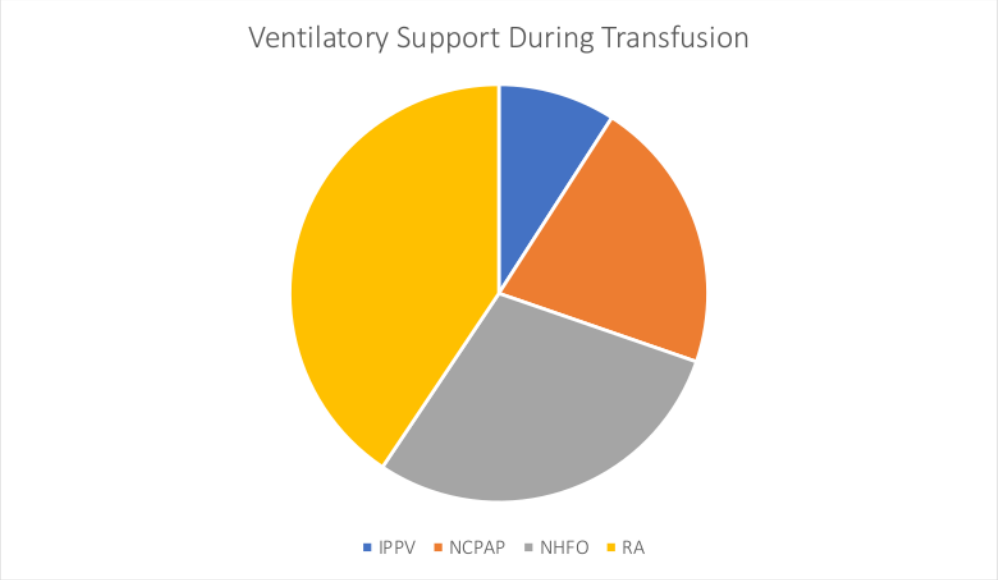

Figure 7: Ventilatory support in Very Low Birth Weight neonates at Pelonomi Tertiary Hospital during RBC transfusion (2012 – 2016)

5.3.2 Universitas Academic Hospital

The median birthweight of the included 476 VLBW neonates at UAH was 1200g, with a median gestational age of 29 weeks. Of these, 257 (54%) had received RBC transfusions. The median birthweight for VLBW neonates who received RBC transfusions was 1160g, with the median gestational age being the same as for the group as a whole at 29 weeks. This indicates that the neonates who received RBC transfusions is representative of the average VLBW neonate treated at UAH (p-value 0,005).

The change in RBC transfusion practices at UAH was less dramatic than at PTH, although a steady decline in the number of transfused VLBW neonates was seen between 2012 and 2016. In 2012, 73 (62%) of the VLBW neonates received RBC transfusions, compared to only 32 (31%) in 2016. Since the number of VLBW neonates managed at UAH stayed relatively constant, between 104 and 117, the change in the number of RBC transfusions can only be explained by a change in transfusion practices in the unit (p-value 0,0003).

The median pre-transfusion haematocrit for these neonates was 0,33 L/L, whilst the median post-transfusion haematocrit was 0,41 L/L. The neonates who were kept NPO during, and for at least 120minutes after the RBC transfusion, was 110 (42,8%).

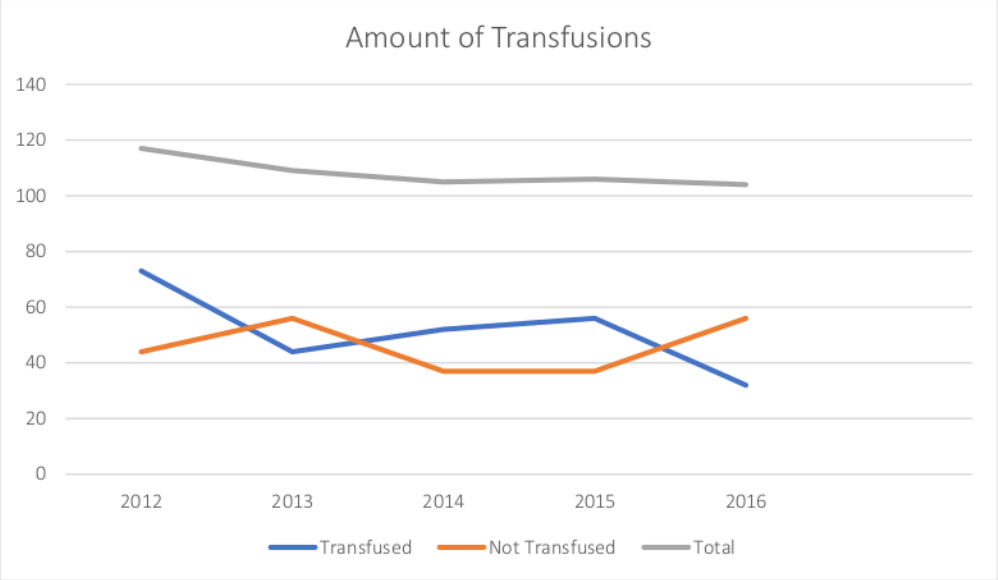

Figure 8: Amount of RBC transfusions in Very Low Birth Weight neonates at Universitas Academic Hospital (2012 - 2016)

The total number of VLBW neonates who received RBC transfusions at UAH dropped slightly from 2012 to 2016, with a percentage rise in the number of neonates who were kept NPO during and after their transfusions. In 2012, 21 (29%) of the 73 transfused neonates were kept NPO during the RBC transfusion, while 14 (44%) of the 32 neonates who were transfused in 2016 were kept NPO during the transfusion. This change in the number of patients kept NPO during and after RBC transfusions over the 5-year period is statistically significant (p-value 0,008).

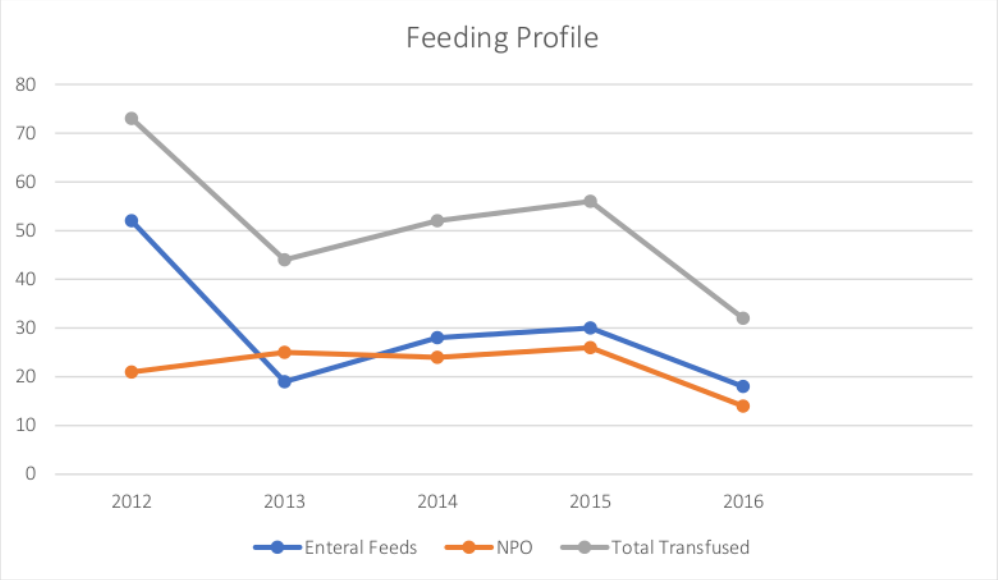

Figure 9: Feeding profile of Very Low Birth Weight neonates transfused at Universitas Academic Hospital (2012 - 2016)

The clinical conditions of neonates whilst receiving RBC transfusions were recorded as follows:

Clinical condition	Number	Percentage
Stable (No clinical abnormality)	155	60,3%
Septic / Septic shock	38	14,8%
Tachycardia	29	11,3%
Respiratory distress	15	5,8%
Hypovolemic shock	11	4,3%
Apnoea	4	1,6%
Tachypnoea	2	0,8%
Exchange transfusion	1	0,4%
Upper Gastro-intestinal Bleed	1	0,4%
Unstable (Specific clinical signs not specified)	1	0,4%

Table 7: Clinical condition of Very Low Birth Weight neonates at Universitas Academic Hospital during RBC transfusion (2012 - 2016)

Again, as at PTH, the majority (60,3%) of the neonates who received RBC transfusions, was recorded to be clinically stable and therefore had no clinical indication for transfusion. This number is similar to the one for PTH. Likewise, the median pre-transfusion haematocrit was the same (0,33L/L), indicating that neonates at UAH received more RBC transfusions than clinically indicated, similar to those at PTH.

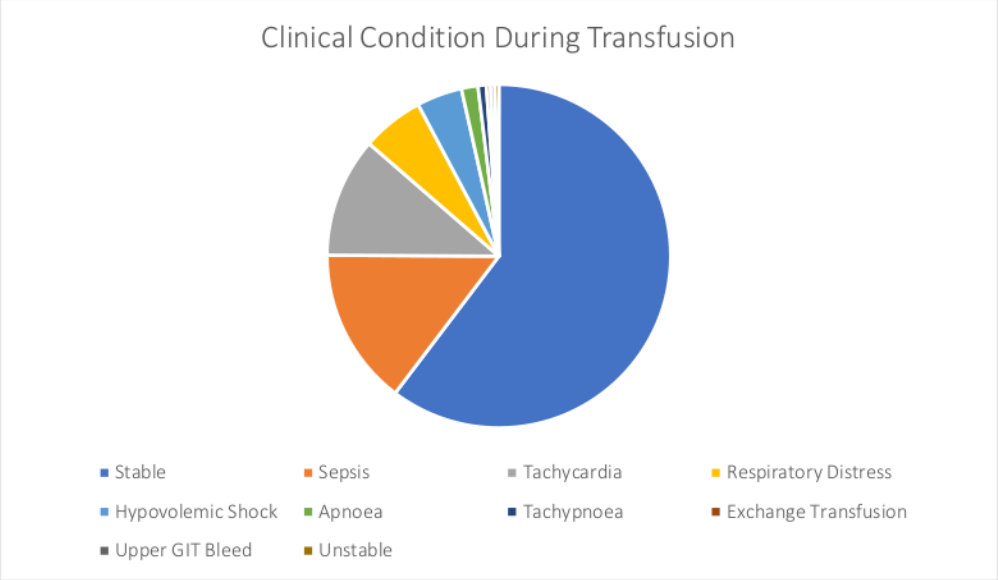

Figure 10: Distribution of clinical condition of Very Low Birth Weight neonates at Universitas Academic Hospital during RBC transfusion (2012 – 2016)

The ventilatory support of the neonates who received RBC transfusions was recorded as follows:

Ventilatory Support	Number	Percentage
High Frequency Oscillatory Ventilation	13	5%
Intermittent Positive Pressure Ventilation	18	7%
Nasal Continuous Positive Airway Pressure	28	11%
Nasal High Flow Oxygen	73	28,4%
Room Air	125	48,6%

Table 8: Ventilatory Support of Very Low Birth Weight neonates at Universitas Academic Hospital during RBC transfusions (2012 – 2016)

The majority of transfused VLBW neonates (48,6%) at UAH had no respiratory problems and were stable on room air. This again points to no clinical indication for RBC transfusion in most of the neonates.

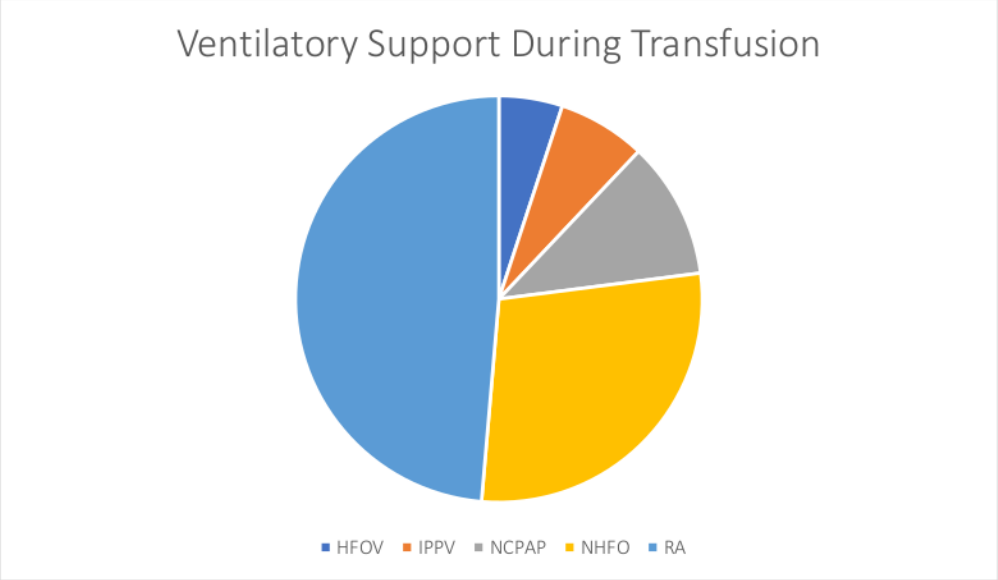

Figure 11: Ventilatory Support of Very Low Birth Weight neonates at Universitas Academic Hospital during RBC transfusions (2012 – 2016)

5.4 NEC results

5.4.1 Pelonomi Tertiary Hospital

Of the 950 VLBW neonates treated at PTH during the 5-year study period, 215 were recorded to have developed NEC. This accounts for an incidence of 22,6%, which is higher than the international standard incidence of 11% in VLBW neonates.[11] Of the 215, 119 (55,3%) developed NEC before receiving an RBC transfusion, while 96 (44,7%) developed TANEC. The majority (43%) of the 215 neonates who developed NEC were classified as Grade I according to the Modified Bell's Staging and did not need surgical intervention. Neonates with NEC Grade III A and B by definition require surgical intervention. Thus, 16,8% of the VLBW neonates who developed NEC required surgical intervention. The Bell's Staging breakdown was as follows.

Grade	Number	Percentage
I	92	43%
IIA	56	26,2%
IIB	30	14%
IIIA	7	3,3%
IIIB	29	13,5%

Table 9: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital (2012 - 2016)

To better understand any changes in the development of the NEC over the five recorded years, the years were looked at individually for severity of the NEC and any patterns that may have flagged throughout the years.

In 2012, 72 of 200 (36%) VLBW neonates developed NEC in PTH. This is quite a high number compared to the international studies. The majority were classified as Grade I (34,7%), and 50 of these (69,4%) had developed TANEC. The total number of neonates who received RBC transfusions in 2012 was 114. Only 45 (39,5%) were kept NPO during and for at least 120minutes after the transfusion.

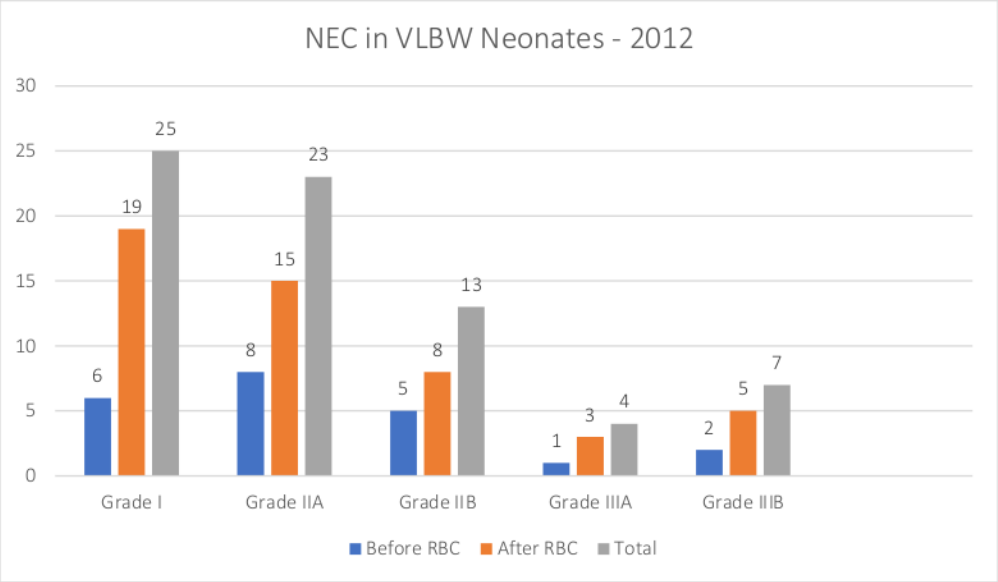

Figure 12: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital – 2012

A minor drop in NEC-cases was seen in 2013 with 52 in total at PTH. Thus, the incidence of NEC in VLBW neonates at PTH in 2013 was 21%, which is lower than 2012, but still higher than expected. The majority of were still classified as Grade I (42,3%). The least frequent Bell's Staging grade was Grade IIIA (2%). There was an accompanying decrease in the percentage of neonates who developed TANEC (25 of the 52: 48%). The total number of neonates who received RBC transfusions dropped from 114 in 2012 (58,5%) to 90 in 2013 (37%), of which 43 (48%) were kept NPO during and for at least 120minutes after the transfusion.

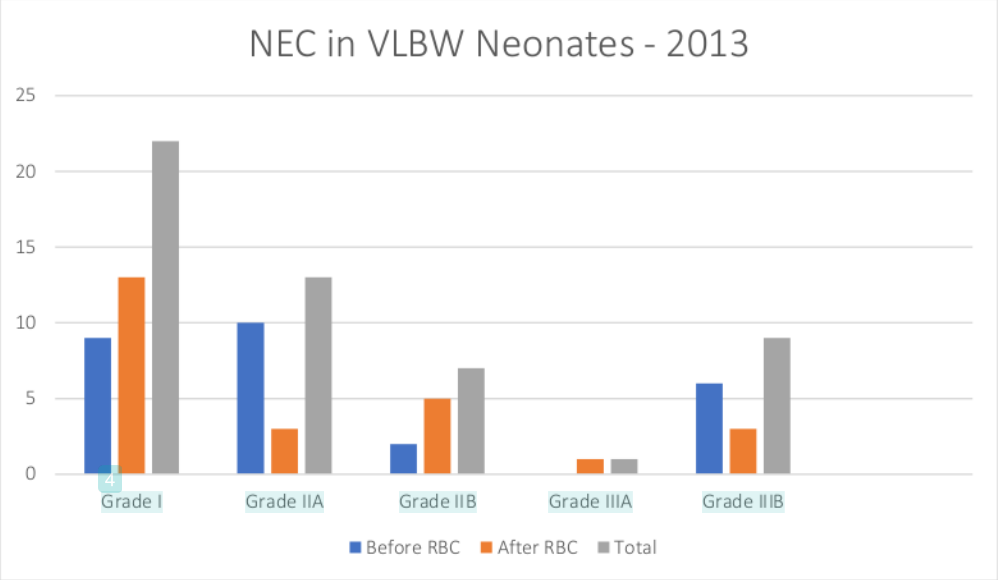

Figure 13: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital – 2013

In 2014 we saw another drop in the total number of VLBW neonates who developed NEC at PTH, when only 27 (13,4%) of the 201 neonates developed NEC. This incidence was much closer to the expected number. The majority were still classified as Grade I (52%), with the least frequent Bell's Staging grade continuing to be Grade IIIA. Of the 27 neonates who developed NEC, only 8 (29,6%) developed TANEC, a drop from the 25 (48%) in 2013. The total number of neonates who received RBC transfusions dropped from 90 in 2013 (37%) to 55 in 2014 (27%), of which 35 (63,6%) were kept NPO during and for at least 120minutes after the transfusion.

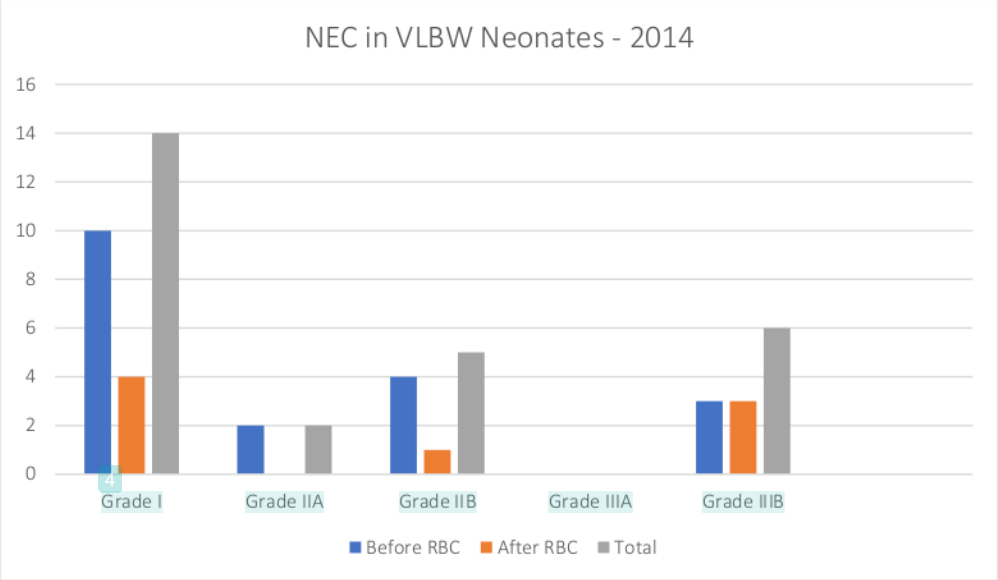

Figure 14: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital - 2014

In 2015, as in 2014, 27(13,3%) of the 203 VLBW neonates developed NEC. Only 3 of these (11%) were TANEC, a further drop from the 8 in 2014 (29,6%). The total number of neonates who received RBC transfusions dropped from 55 in 2014 (27,4%) to 44 in 2015 (22%), continuing the persistent drop from 2012. Twenty-six (60%) were kept NPO during and for at least 120minutes after the transfusion.

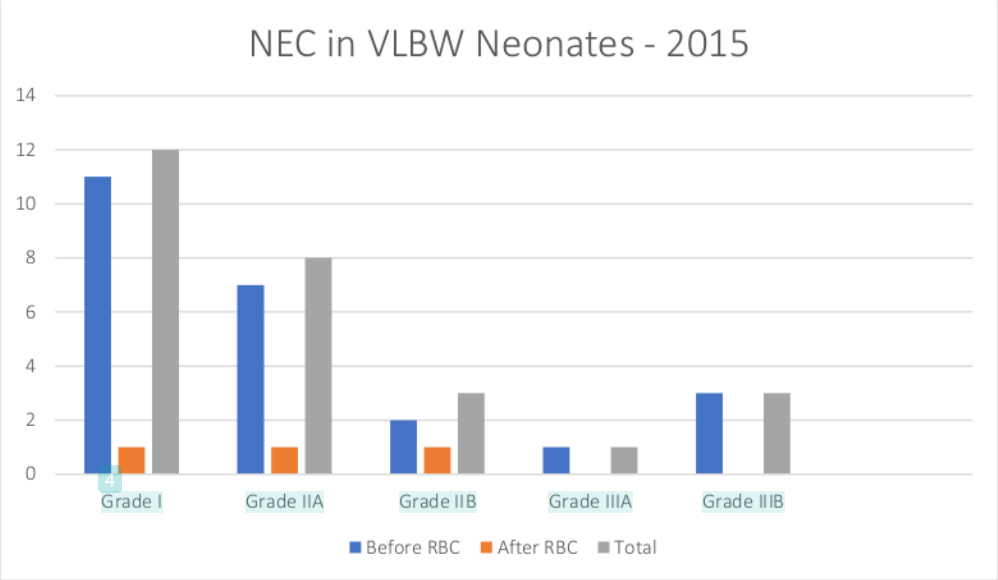

Figure 15: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital – 2015

2016 brought a slight increase in the number of VLBW neonates who developed NEC in PTH, when 36 (18,4%) of the 196 patients developed NEC. Of the 36 neonates who developed NEC, only 7 (20%) had developed TANEC, the percentage of al 5 study years. The total number of neonates who received RBC transfusions dropped from 44 in 2015 (22%) to 37 in 2016 (19%). Of those neonates who received RBC transfusions, 23 (62%) were kept NPO during and for at least 120minutes after the blood transfusion.

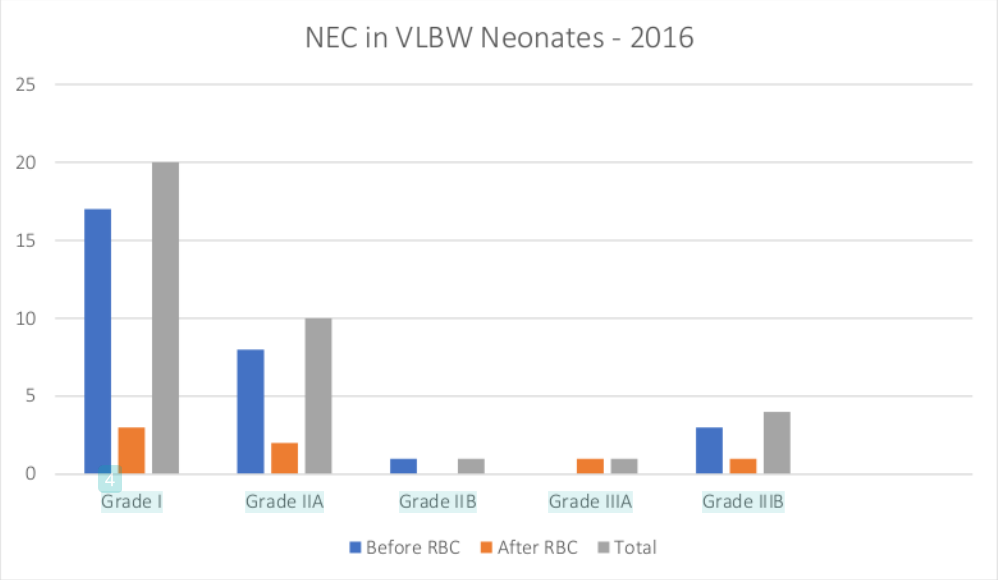

Figure 16: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Pelonomi Tertiary Hospital – 2016

Even though the incidence of NEC in VLBW neonates in PTH (22,6%) is higher than the international standard of 11% [11], a remarkable decrease was seen in the overall numbers over the 5-year study period. The majority of the neonates had developed NEC Grade I or II.

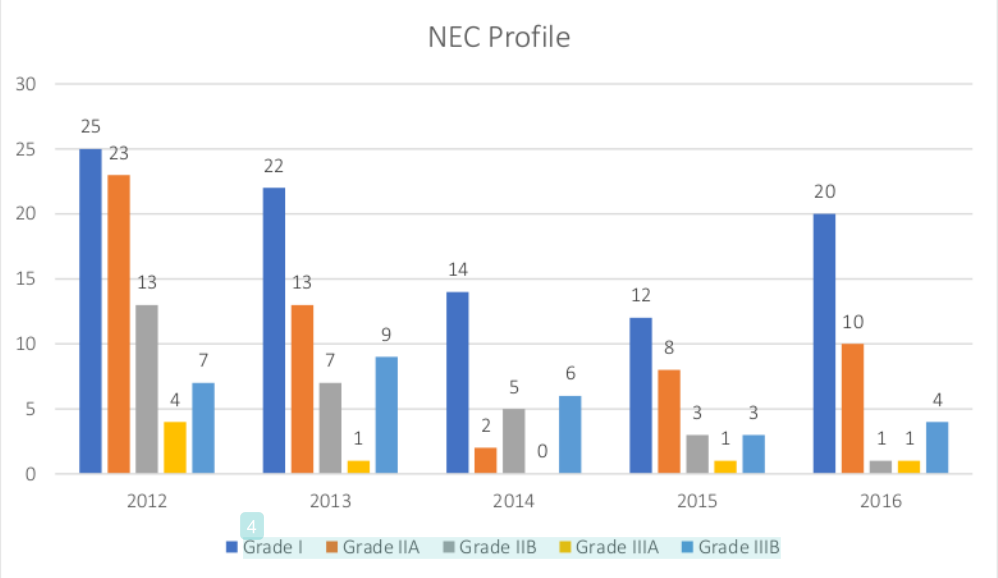

Figure 17: Modified Bell's Staging profile for Very Low Birth Weight neonates who developed NEC at Pelonomi Tertiary Hospital (2012 - 2016)

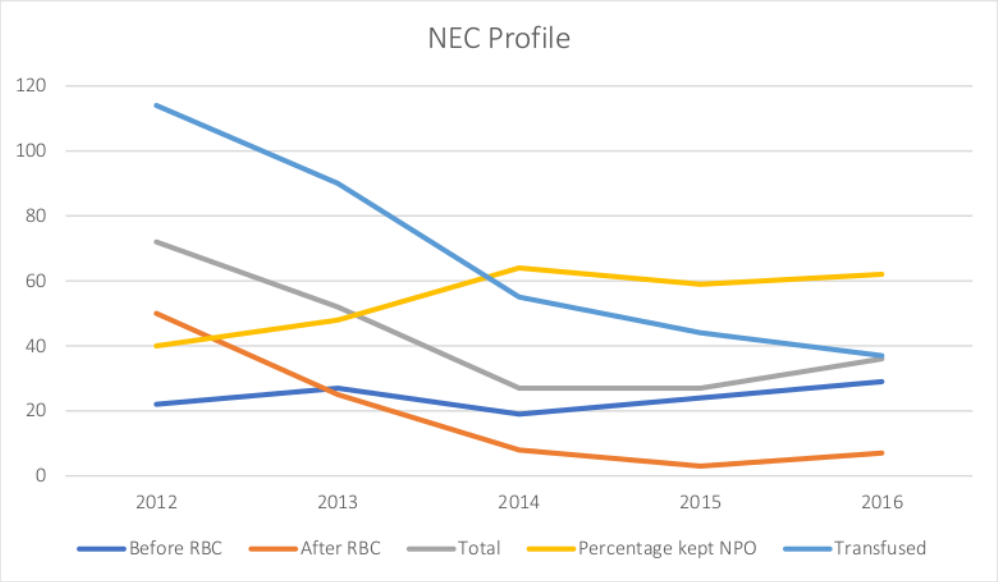

Figure 18: Profile of Very Low Birth Weight neonates who developed NEC at Pelonomi Tertiary Hospital (2012 - 2016)

Over the 5-year study period a decreased number of RBC transfusions were given to VLBW neonates, with an increased number of them kept NPO during transfusions. This coincided not only with a decrease in the incidence of TANEC in our VLBW population, but also with a decrease in the total number of VLBW neonates who developed NEC. Thus, a relative increase in the number of neonates kept NPO during RBC transfusion (40% in 2012 vs. 62% in 2016), coincided with a decrease in the total number of neonates transfused (114 in 2012 vs. 37 in 2016, p-value 0,03). This again coincided with a decrease in the number of neonates who developed TANEC (50 in 2012 vs. 7 in 2016, p-value 0.002). This correlation is of statistical significance (p-value 0,046).

5.4.2 Universitas Academic Hospital

Of the 476 VLBW neonates treated at UAH during the 5-year study period, 149 (31,3%) were recorded to have developed NEC. This accounts for 31,3% of the VLBW neonates, placing our incidence far above the international standard of 11% [11]. Of these, 78 (52,3%) neonates developed NEC before receiving an RBC transfusion, and 71 (47,7%) developed TANEC. The majority (43,6%) was classified as Grade I according to the Modified Bell's Staging. Neonates with NEC Grade III A and B by definition require surgical intervention. Thus, 11,4% of the VLBW neonates who developed NEC required surgical intervention. The Bell's Staging breakdown was as follows:

Grade	Number	Percentage
I	65	43,6%
IIA	45	30,2%
IIB	22	14,8%
IIIA	2	1,3%
IIIB	15	10,1%

Table 10: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital (2012 - 2016)

To better understand any changes in the development of NEC over the five recorded years, the years were looked at individually for severity of the NEC, and any specific patterns that may have flagged throughout the years.

In 2012, a total of 38 (32,5%) VLBW neonates developed NEC at UAH. The majority were classified as Grade IIA (50%). Thirty-one (81,6%) had developed TANEC. In 2012, the number of VLBW neonates who received RBC transfusions was 73 (62,4%), of which only 21 (28,8%) were kept NPO during and for at least 120minutes after the transfusion. Thus, more than half of the VLBW neonates received RBC transfusion, whilst less than a third where kept NPO during and after the transfusion, and more than 80% of them subsequently developed TANEC.

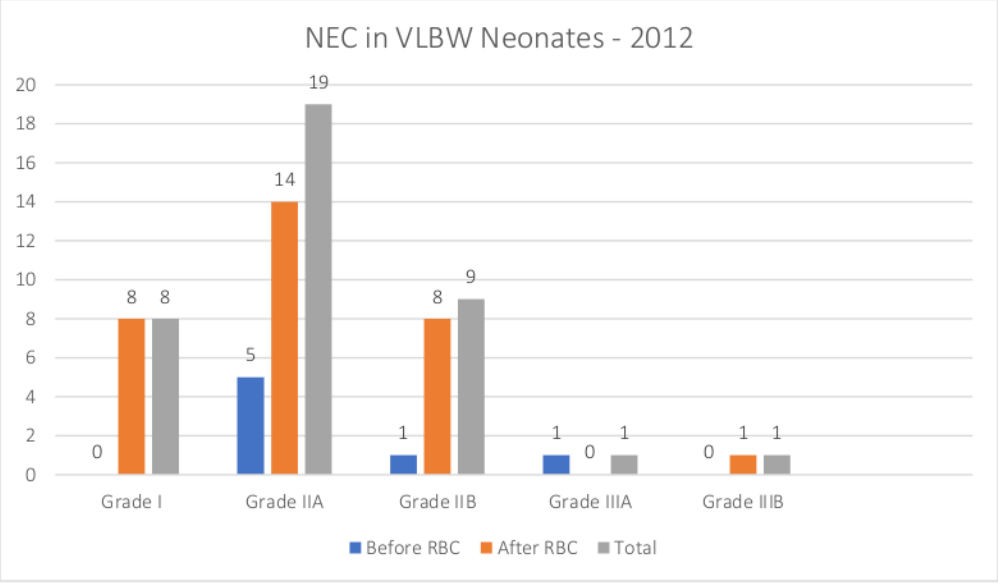

Figure 19: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2012

During 2013, 31 (28,4%) of 109 VLBW neonates developed NEC at UAH, comparing well with the 38 (32,5%) of 2012. The majority of these were classified as Grade I (45%), with none classified as Grade IIIA. Twelve of the 31 (38,7%) had developed TANEC, a major drop from the 31 (81,6%)

in 2012. The total number of neonates who received RBC transfusions declined from 73 in 2012 (62,4%) to 44 in 2013 (40,4%), of which 25 (57%) were kept NPO during and for at least 120minutes after the transfusion. This is a good increase from 2012, as only 28,8% of the neonates who received RBC transfusions in 2012 were kept NPO during and for at least 120min after the transfusion.

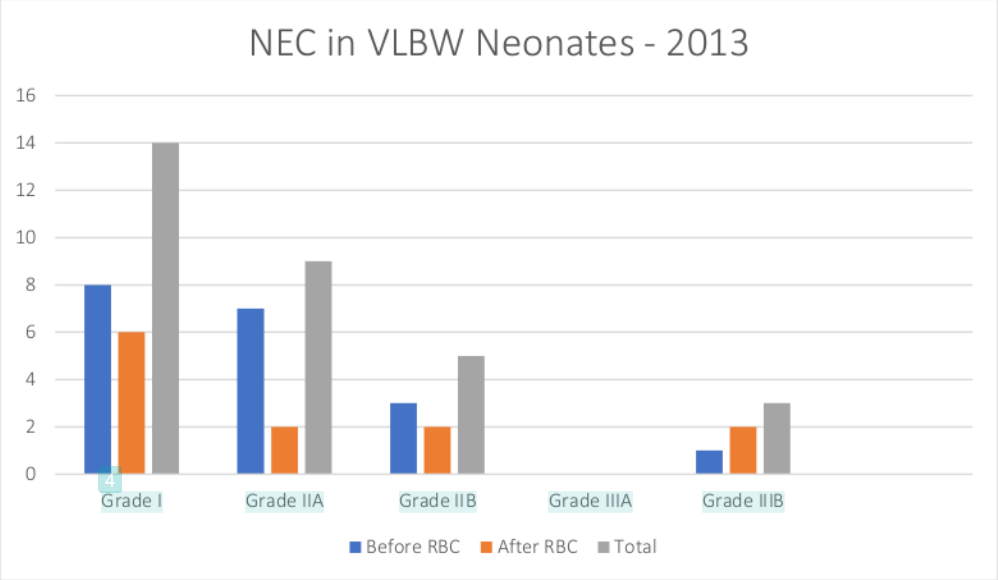

Figure 20: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2013

2014 saw another slight drop in the number of VLBW neonates who developed NEC at UAH, when only 26 (25%) of the 105 VLBW neonates developed NEC. The majority of these were still classified as Grade I (46%), with the least frequent Bell's Staging grade continuing to be Grade IIIA. Of the 26 neonates who had NEC, 12 (46%) had developed TANEC. The total number of neonates who received RBC transfusions increased slightly from 44 in 2013 (40,4%) to 52 in 2014 (49,5%). Of these 24 (46,2%) were kept NPO during and for at least 120minutes after the transfusion.

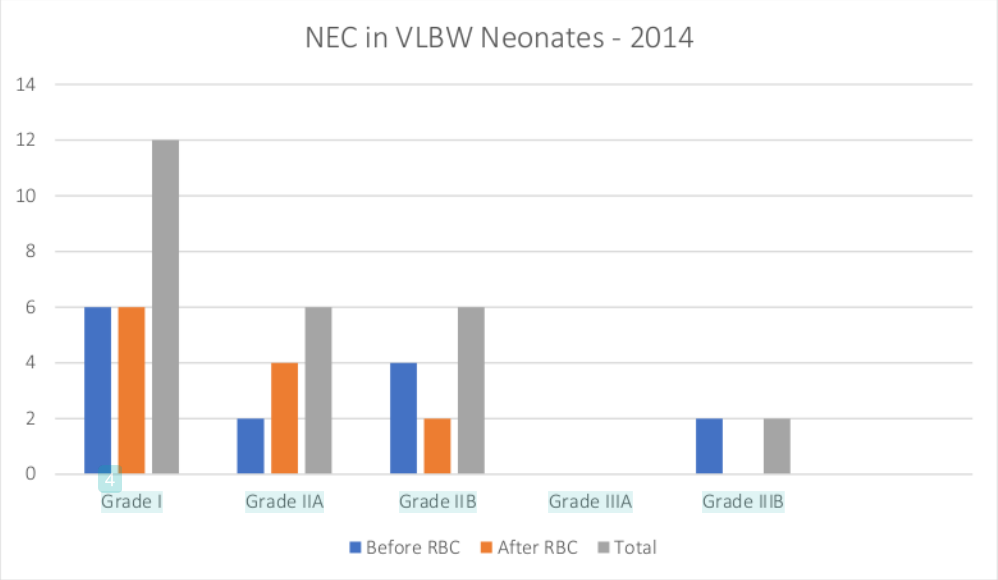

Figure 21: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2014

In 2015, 27 (29%) of the 93 VLBW neonates treated at UAH developed NEC. Of these, 10 (37%) had developed TANEC, with a significant percentage drop from the 12 in 204 (46%). The total number of neonates who received RBC transfusions increased again from 52 in 2014 (49,5%) to 56 in 2015 (60%). Of these, 26 (46,4%) were kept NPO during and for at least 120minutes after the transfusion.

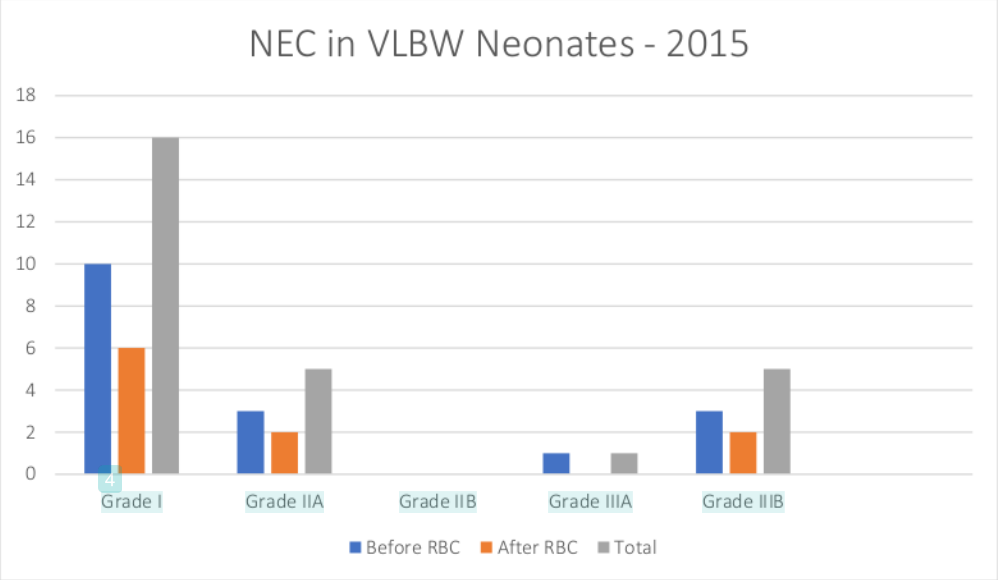

Figure 22: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2015

Lastly, in 2016 a persistent number of 27 (26%) VLBW neonates developed NEC at UAH. Of the 27 VLBW neonates who developed NEC, only 6 (22%) had developed TANEC. This is a very large drop from the 81,6% in 2012. The total number of neonates who received RBC transfusions again dropped from 56 in 2015 (52,8%), to 32 in 2016 (30,8%). Of these VLBW neonates who received transfusions, 14 (44%) were kept NPO during and for at least 120minutes after the transfusion.

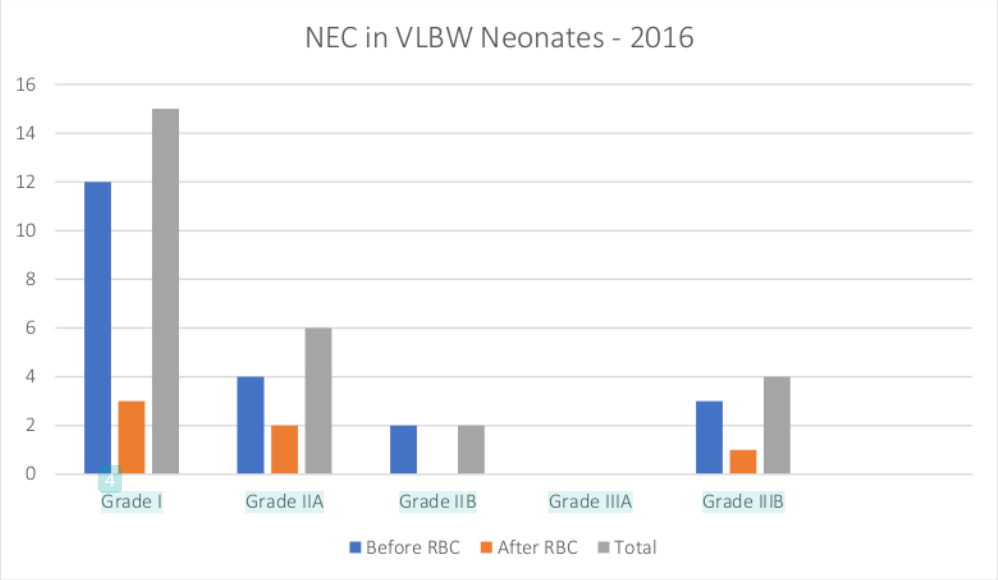

Figure 23: Modified Bell's Staging breakdown for Very Low Birth Weight neonates at Universitas Academic Hospital – 2016

Thus, a relative increase in the number of neonates kept NPO during RBC transfusion (29% in 2012 vs. 44% in 2016), coincided with a decrease in the total number of neonates transfused (73 in 2012 vs. 32 in 2016, p-value 0,008). This also coincided with a significant decrease in the number of neonates who developed TANEC (31 in 2012 vs. 6 in 2016, p-value 0,0005). However, the correlation between neonates kept NPO during RBC transfusion, and those who developed TANEC at UAH, was not statistically significant (p-value 0,1673).

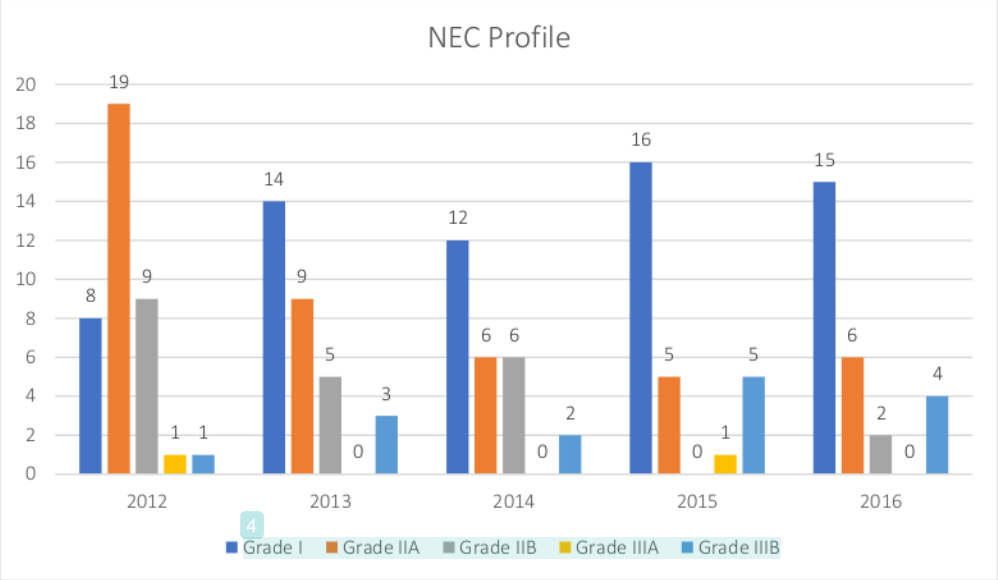

Figure 24: Modified Bell's Staging Profile for Very Low Birth Weight neonates who developed NEC at Universitas Academic Hospital (2012 - 2016)

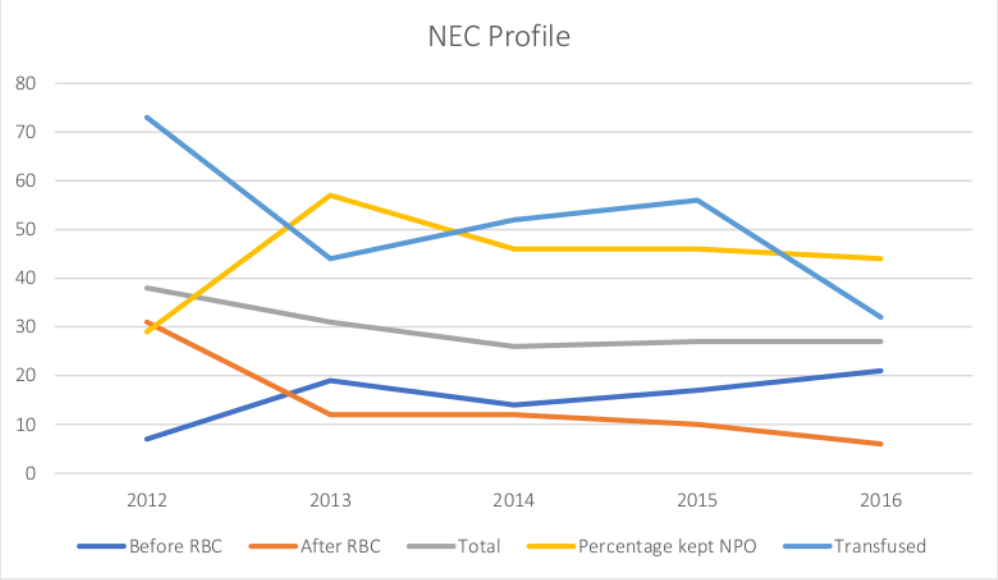

Figure 25: Profile of Very Low Birth Weight neonates who developed NEC at Universitas Academic Hospital (2012 - 2016)

Chapter 6: Discussion

Necrotizing enterocolitis (NEC) is one of the greatest disease burdens faced in neonatal units, and the most common acquired gastrointestinal pathology encountered in Very Low Birth Weight (VLBW) neonates. Despite the high incidence and subsequent morbidity and mortality associated with this disease, there is still a great paucity in our knowledge regarding its aetiology and pathology.

This lack of knowledge is not due to a lack of research in the field, as can be seen by the multitude of research efforts that has been made in the last 40 years. Research is especially critical in units with a high incidence of NEC, where the current disease burden should be utilized to further our knowledge of the pathophysiology and enable better management and hopefully prevention of this devastating disease.

6.1 Epidemiological data

The number of VLBW neonates treated at both Pelonomi Tertiary Hospital (PTH) and Universitas Academic Hospital (UAH), stayed relatively constant from 1 January 2012 to 31 December 2016. Given this fact, any changes in the incidence of NEC and associated pathology can be attributed to a change in intervention practices, rather than a change in the patient population. The number of VLBW neonates treated in both these academic hospitals also served as a large enough study sample for the purposes of this study.

The VLBW neonates treated at UAH had a median birth weight of 1200g, 100g less than the median birthweight of neonates treated at PTH. The median gestational age of VLBW neonates at UAH was also slightly lower than that of the VLBW neonates at PTH, 29 weeks and 30 weeks, respectively. This indicates that the VLBW neonate population at both hospitals are similar enough to allow comparison.

The prevalence of congenital abnormalities was determined before applying the exclusion criteria for the study, with 1.2% and 3.9% of the neonates excluded at PTH and UAH, respectively. The incidence of significant congenital abnormalities in our VLBW neonates was 2,1%. The most common surgical congenital abnormality in VLBW neonates was duodenal atresia (0,44%) while oesophageal atresia (with or without tracheo-oesophageal fistulae) was the second most common with an incidence of 0,25%. The most common non-surgical congenital conditions were Trisomy 21, and Trisomy 18. There are very few recent studies looking specifically at the incidence of congenital surgical abnormalities in VLBW neonates specifically, so we were not able to correlate our findings with any other significant literature. The number of neonates excluded from the study did not significantly decrease the number of neonates that were included in the study.

6.2 RBC transfusions

Given the fact that the numbers in our VLBW neonatal population did not change much over the 5-year study period, any changes in RBC transfusion numbers can directly be linked to changes in

transfusion practices in the units. However despite these changes in transfusion practices, the majority of VLBW neonates who received RBC transfusions in these two academic hospitals were transfused at higher haematocrits (median 33%) than the current international guidelines,[73] as well as during stable clinical conditions. According to these guidelines, neonates with a haematocrit of between 0,28 and 0,33 should only be transfused if they are clinically unstable (Table 11), while about 60% of the neonates transfused in both of our institutions were recorded to have been stable before the transfusion, and more than 40% of them was on room air, not indicating any respiratory distress. This is not only an expensive practice but could also indicate potential waste of a valuable resource in the form of donor blood. It is therefore mandatory that a closer look be taken at the local RBC transfusion practices. The acceptance or compilation of a scientific protocol at these neonatal units to decrease the amount of unnecessary RBC transfusions, should be priority.

Indications for Transfusion of RBC's in Neonates	
HCT	**AGE and CLINICAL CONDITION**
<35%	≤1week of Life **AND** Unstable*
<28%	≤1week of Life **OR** Unstable*
<20%	>1week of Life **AND** Unstable*
*Unstable clinical condition is defined as a state of poor oxygen delivery, e.g. Respiratory distress/apnoea, Cardiovascular deterioration etc.	

Table 11 : Indications for RBC Transfusions in neonates [73]

Two separate studies pointed towards a relationship between RBC transfusions at lower haematocrit levels, and the development of TANEC [8875]. A study similar to this one done in 2015 showed a significantly lower pre-transfusion haematocrit for their patients who had developed TANEC (0,24L/L).[75] In a study on the effects of RBC transfusions in VLBW neonates done by Valieva et al in 2009, their patients had a mean pre-transfusion haematocrit of only 0,28L/L.[73] The median pre-transfusion haematocrit of 0,33L/L at the units in our study, is therefore much higher than those in the current literature.

There was a steady decrease in the number of VLBW neonates who received RBC transfusions in both of our local hospitals over the five years study period, pointing towards a definite change in transfusion practices. The total number of VLBW neonates who received RBC transfusions at PTH persistently dropped from 114 (57%) in 2012, to 37 (18,8%) in 2016. During the same time at UAH the numbers declined from 73 (62%) in 2012, to 32 (31%) in 2016. Despite the high local RBC transfusion rates, it still undercuts most of the large international studies done on transfusions in neonates, where transfusion rates between 66% and 90% have been recorded.[73,89]

Regarding exchange transfusions, only 9 patients at UAH and PTH needed an exchange transfusion during the 5-year study period. Al of them were kept NPO during and for at least 120min after transfusion since exchange transfusion protocols are more well established in our units. Only 3 (33%) of the VLBW neonates who had received exchange transfusions, subsequently developed TANEC, but all of them developed Grade IIIB NEC and needed surgical intervention.

A positive change in RBC transfusion practices was seen, as an increasing percentage of the VLBW neonates receiving RBC transfusions were kept NPO during, and for at least 120minutes after the transfusion, in response to the latest publications on TANEC. In 2012 only 39,5% of the VLBW neonates who received RBC transfusions at PTH were kept NPO during the transfusion. This figure steadily rose over the 5-year period, reaching a high of 62% in 2016.

A similar picture could be seen at UAH where only 28,8% of VLBW neonates who received RBC transfusions were kept NPO in 2012, while 44% were kept NPO in 2016. This indicates progress. However, this is still not adequate compared to other studies where up to 83% of neonates who received RBC transfusion were kept NPO.[71,89]

6.3 Necrotising Enterocolitis

Even though the incidence of NEC in VLBW neonates the two academic units in Bloemfontein (25,5%) is higher than the international standard of 11%, there has been a great decrease in the overall incidence of NEC over the 5-year study period, with the vast majority of the neonates developing Grade I (43,5%) or Grade II (41,3%) NEC.

A study conducted by Singh et al. in 2011 found the incidence of NEC in VLBW neonates in their institution to have been 3,67%. This is much lower than our incidence of 22,6% in PTH, and 31,3% at UAH, with a combined overall incidence of 25,5%.[75]

Regarding surgical intervention for NEC, 16,8% and 11,4% of VLBW neonates with NEC at PTH and UAH respectively required surgical input. This is much lower than the estimates found in the literature where 35 – 50% of neonates with NEC required surgical intervention.[15,17,18] This significantly lower percentage in our study can be attributed to possible over diagnosing of lower grades of NEC in neonates, skewing the numbers in favour of non-surgical NEC.

It is important to note that neonates who require surgical intervention are transferred to UAH from PTH, and thus care was taken during the data collection to not duplicate the patients who were initially diagnosed at PTH, but subsequently transferred to UAH. All neonates who were diagnosed with NEC at PTH was only included in the PTH data collection, even if they were transferred to UAH for surgical intervention.

There is a statistically significant correlation between the change in numbers of neonates who received RBC transfusions, and the number of neonates who developed TANEC in PTH (p-value 0,0005) and UAH (p-value 0,0005) respectively, as well as for the combined VLBW population (p-value 0,0005).

A statistically significant correlation was found between the number of VLBW neonates who developed TANEC, and those who were kept NPO during and after their RBC transfusions at PTH (p-value 0,046), but not for the VLBW neonates at UAH (p-value 0,1673). This leads to the overall VLBW population not having a significant correlation between the change in those who were kept NPO, and those who developed TANEC (p-value 0,078).

6.3.1 Pelonomi Tertiary Hospital

In 2012 the practice of keeping patients NPO during RBC transfusions had not been thoroughly established yet. This could account for the fact that more than half of the neonates who developed NEC had done so after an RBC transfusion (69,4%). In 2013 the number of neonates not kept NPO during RBC transfusions was still quite high (48%).

In 2014 an increased number of neonates where kept NPO during RBC transfusions (63,7%). At the same time, a decrease in the number of neonates who developed TANEC was noted (29,6%).

Similarly, in 2015 a drop in the total number of neonates who received RBC transfusions was recorded, with only 11,1% who developed NEC within 48-hours after receiving an RBC transfusion. Another increase in the number of patients kept NPO during their RBC transfusion (60%) coincided with this decrease in TANEC, which supports the hypothesis that keeping VLBW neonates NPO during RBC transfusions will lead to a lower incidence of TANEC.

Thus, a relative increase in the number of neonates kept NPO during RBC transfusion (40% in 2012 vs. 62% in 2016), coincided with a decrease in the total number of neonates transfused (114 in 2012 vs. 37 in 2016, p-value 0,03). This also coincided with a decrease in the number of neonates who developed TANEC (50 in 2012 vs. 7 in 2016, p-value 0,002). This correlation is of statistical significance (p-value 0,046).

6.3.2 Universitas Academic Hospital

Between 2012 and 2013 a change in the RBC transfusion practices at UAH could already be seen as the number of neonates who received RBC transfusions dropped from 73 (62,4%) in 2012, to 44 (40,3%) in 2013. More neonates were also kept NPO during their RBC transfusions, with only 28,8% kept NPO in 2012 versus 57% kept NPO in 2013. This change in transfusion practices coincided with a drop in the number of neonates who developed TANEC, namely 81,6% in 2012 versus 38,7% in 2013.

2014 brought a slight increase in the number of RBC transfusions and a decreased percentage of them were kept NPO during the transfusions. With 49,5% of the VLBW neonates who received RBC transfusions, and only 46,2% kept NPO. This coincided with a percentage increase in the number of neonates who developed TANEC, supporting the theory that feeds during RBC transfusions increases the risk for VLBW neonates developing TANEC.

Finally, a drop in the number of VLBW neonates who received RBC transfusions was seen in 2016, with the lowest percentage recorded at UAH over the study period of 30,8%. However, the percentage of these neonates who were kept NPO during their RBC transfusion dropped to 44%, and the number of VLBW neonates who developed TANEC also dropped to 22%.

A relative increase in the number of neonates kept NPO during RBC transfusion (29% in 2012 vs. 44% in 2016), coincided with a decrease in the total number of neonates transfused (73 in 2012 vs. 32 in 2016, p-value 0,008), which also coincided with a decrease in the number of neonates who developed TANEC (31 in 2012 vs. 6 in 2016, p-value 0,0005). However, the correlation between neonates who were kept NPO during transfusion, and those who developed TANEC, was not statistically significant at UAH (p-value 0,1673).

6.4 Limitations of this Study

Potential limitations of this study include its retrospective uncontrolled design. Some information might have been missed by the treating physicians at the time of hospital stay, and not included in the online Meditech© notes, and would thus not have been accessed.

Chapter 7: Conclusion and Suggestions

Necrotising enterocolitis is by far the largest surgical and gastro-intestinal burden faced by neonatologists and paediatric surgeons in Academic Hospitals in Bloemfontein. The local incidence is much higher than the global accepted average, necessitating a greater responsibility to find ways in which to improve the current management practices, and implement preventative protocols. It is the leading cause of surgical deaths in our local VLBW population, by far outnumbering the amount of other congenital surgical abnormalities seen in the neonatal population. Despite a gradual decline seen in the number of VLBW neonates who developed NEC over our study period, more needs to be done to further the progress. NEC is also a disease that causes a multitude of long-term complications, including short bowel syndrome, and poor neurological outcomes.[29,87]

As with most medical conditions, initially the focus of care was on managing the NEC once it has already developed. Medical knowledge has however moved on to a more advanced era, where doctors caring for neonates, are no longer merely interested in managing the problems once they arise. On the contrary, they are increasingly interested in preventing the conditions from arising in the first place. Multiple new strategies are being suggested to downregulate the risk that premature neonates carry to develop NEC. By far the most important of these is the practice of exclusive breastmilk feeding. This is a free resource which has been proven repeatedly by studies to carry a large preventative benefit to premature neonates against the development of NEC. Especially in low to mid-income countries the value of breastfeeding cannot be emphasized enough, and the promotion of breastfeeding should be a key component in our neonatal care.

Other novel interventions for the prevention of NEC, such as the Ganz protocol[56], have shown promise, but need further studies to recreate their results before it can be adopted as a recommendation. As seen from our study, the neonatal intensive care units in Bloemfontein's Academic Hospitals manage enough VLBW neonates who develop NEC, that an interventional study testing the use of an oral antibiotics and probiotic protocol could be of great scientific gain. Since the exact aetiology of NEC is still unknown, further studies are necessary to find possible causes, as well as contributing factors, and our units could render large amounts of scientific evidence if our patient data is utilized correctly.

The authors are of the opinion that a strict protocol for RBC transfusion in neonates should be established at all hospitals. The changes in RBC transfusion practices witnessed over the 5-year period in the two academic hospitals in Bloemfontein, Free State, proved a definite correlation between RBC transfusions in VLBW neonates, and the development of TANEC (NEC within 48hours after the transfusion). Even though the steady decrease in numbers of VLBW neonates

transfused over the 5-year period, already indicates a move in the right direction, an unacceptable number of neonates are still transfused without any clinical indication, causing an unnecessary risk for development of NEC and other RBC transfusion related morbidities.

Looking at the results of this data collection, one can postulate that an improvement in RBC transfusion practices, by means of a protocol, will lead to not only preventing unnecessary transfusions, thus saving blood products, a limited resource in this country, and money but also protecting at risk neonates from developing a potentially fatal disease.

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