Prevalence of thrombocytopaenia in neonates admitted in neonatal high care unit of Pelonomi Tertiary Hospital Bloemfontein between January 2017 and June 2017

By

Dr Anselm Uche Onwugbolu, MBBS

Submitted in fulfilment of the requirements for the degree
Master of Medicine (MMed) in Paediatrics

in the

Department of Paediatrics and Child Health
Faculty of Health Sciences at the University of the Free State

Submission Date: 12 August 2019

CANDIDATE

Dr Anselm Uche Onwugbolu, MBBS
Registrar: Department of Paediatrics and Child Health
Faculty of Health Sciences
University of the Free State
Student number:
2016433324

STUDY LEADER

Dr J van Rooyen MBchB, MMed (Paeds), FC Paed(SA)
Consultant: Department of Paediatrics and Child Health
Faculty of Health Sciences
University of the Free State

DECLARATION:

I, Dr Anselm Uche Onwugbolu, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification in Paediatrics at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

AUTHOR'S CONTRIBUTIONS:

The contribution of each author of the article is stipulated below:

Author	Affiliation	Role in the study
Onwugbolu, A U.	Department of Paediatrics and Child Health Faculty of Health Sciences University of the Free State	Study conception and design, acquisition of data, interpretation of data, drafting the article
Van Rooyen, J.J.	Department of Paediatrics and Child Health Faculty of Health Sciences University of the Free State	Supervision, study conception and design, revising critically for clinical content, give final approval of the version to be submitted
Bouwer, A.S.	Department of Paediatrics and Child Health Faculty of Health Sciences University of the Free State	Design of the manuscript, drafting the article, revising critically
Van Rooyen, C.	Department of Paediatrics and Child Health Faculty of Health Sciences University of the Free State	Analysis of data

All researchers declare that they have no conflict of interest and that no other situation of real, potential or apparent conflict of interest is known to them. They undertake to inform the University of any change in these circumstances.

ACKNOWLEDGEMENTS:

I wish to sincerely convey my gratitude and appreciation to the following persons who assisted me with the completion of this study:

- My study leader, Dr J van Rooyen, MBchB, MMed (Paeds), FC Paed (SA)
  Consultant: Department of Paediatrics and Child Health, Faculty of Health Sciences, University of the Free State, for his passion and enthusiasm in the field of academic research; and for his mentorship, guidance, support and unwavering faith in me against all odds.
- My research coordinator, Mrs A Bouwer, Research Coordinator: Department of Paediatrics and Child Health, Faculty of Health Sciences, University of the Free State, for her optimism, encouragement, unfailing patience, guidance, support and attention to details and quality assurance aspects of the study.
- Prof. André Venter, Head of the Department of Paediatrics and Child Health, Faculty of Health Sciences, University of the Free State for his fatherly support, guidance and personal review of my protocol.
- Mr. Cornel van Rooyen, Researcher: Biostatistics, Faculty of Health Sciences, University of the Free State, for his astute biostatistical expertise and his assistance in the statistical analysis of the study.
- And lastly, but definitely not the least, to my lovely family- my wife, Mrs Miriam CN Onwugbolu, for her undying love, multiple selfless sacrifices, and countless abdications from her own needs for my own success; and to our lovely kids- Philomena, Glory and Chichi for their understanding and show of supports in their own little ways.

TABLE OF CONTENTS:

LIST OF ACRONYMS:

AGA	Appropriate-for-Gestational Age
APH	Antepartum Haemorrhage
DIC	Disseminated Intravascular Coagulation
ELBW	Extremely Low Birth Weight
EOTP	Early Onset Thrombocytopaenia
FBC	Full Blood Count
FNAIT	Foetal and Neonatal Alloimmune Thrombocytopaenia
GA	Gestational Age
GDM	Gestational Diabetes Mellitus
HELLP	Haemolysis, Elevated Liver enzymes and Low Platelet count
HIV	Human Immunodeficiency Virus
HPA	Human Platelet Antigen
ICH	Intracranial Haemorrhage
ITP	Immune Thrombocytopaenic Purpura
IUGR	Intrauterine Growth Restriction
IVH	Intraventricular Haemorrhage
LGA	Large-for-Gestational Age
LOTP	Late Onset Thrombocytopaenia
NEC	Necrotizing Enterocolitis
NHCU	Neonatal High Care Unit
NHLS	National Health Laboratory Service
NICU	Neonatal Intensive Care Unit
PET	Pre-Eclamptic Toxaemia. Also known as Pre-Eclampsia
PIH	Pregnancy Induced Hypertension
PROM	Premature Rupture of the Membrane
SGA	Small-for-Gestational Age
TAR	Thrombocytopaenia Absent Radii
TORCH	Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19, Rubella, Cytomegalovirus (CMV) and Herpes infections
UFS	University of the Free State
VLBW	Very Low Birth Weight

SELECTED DEFINITIONS AND TERMS:

Appropriate for gestational age	A birth is considered to be appropriate for gestational age if the birth weight is between the 10th and 90th percentiles for the infant's gestational age on the Lubchenco infant's chart (24).
Birth Asphyxia	Birth asphyxia occurs when at least three of the following criteria apply: (a) signs of foetal distress before delivery (abnormal cardiotocography recording), (b) Apgar score ≤5 at 5min, (c) arterial pH <7.1 and base excess ≤-16mmol/L or lactate >10mmol/L in either arterial umbilical cord blood sample or capillary blood gas within one hour after birth, (d) respiratory failure requiring resuscitation during at least 5min after birth, (e) multiple organ failure (17).
Extremely low birth weight infants	Infants born with birth weight less than 1000 grams (14,25).
Intrauterine growth restriction	A reduction in the expected foetal growth of an infant. A foetus $<10^{th}$ weight percentile for age or a ponderal index <10%. SGA and IUGR are related but not synonymous (26).
Neonate	Baby at the first twenty-eight days of life (24).
Pre-Eclampsia	Hypertension with proteinuria in pregnant women after 20 weeks' gestation (26).
Premature infants	Infants born before the start of 37 weeks' gestation (259 days) (24).

Small for gestational age	Birthweight 2 standard deviations below the mean weight for gestational age or below the 10^{th} percentile for gestational age (13).
Very low birth weight infants	Infants born with weight less than 1500 grams (13,14).

LIST OF TABLES:

LIST OF FIGURES:

ABSTRACT:

BACKGROUND: Thrombocytopaenia is the most common haematological abnormality, besides anaemia, found in the neonates admitted to a neonatal intensive care unit. Even though most cases are mild or moderate, requiring minimal or no intervention, and resolving spontaneously within the first week of life; a few infants, especially the very sick ones or the extremely low birth weights (<1000g), may present with severe and in some cases persistent thrombocytopaenia, which may require intervention such as platelet transfusion.

OBJECTIVES: The primary objective of this study was to determine the prevalence of thrombocytopaenia in neonates admitted to the neonatal high care unit (NHCU) of Pelonomi Tertiary Hospital (PTH).

METHODS: The study was a retrospective descriptive study conducted on 694 infants admitted to the NHCU of PTH between January 2017 and June 2017. Data included were neonatal demographic information, information regarding the clinical data of neonate and the mother obtained from the neonatal admission book used routinely at PTH by the admitting medical doctor. Infants with thrombocytopaenia were identified using the National Health Laboratory Service web results viewer. The data regarding the rate of performance of platelet count per patient in the cohort group was not collected as the primary objective was to determine whether or not thrombocytopaenia was present in the samples taken within the 72 hours of birth. Also, the common reason for doing blood investigations in the cohort group was not specified during the data collection because this attribute was not documented in the patients' records. The data analysis was performed using the SAS System version 9.4.

RESULTS: During the study period, a total of 694 neonates were admitted to the NHCU of PTH. Of these, 16.3% (n=113) infants were diagnosed with thrombocytopaenia (platelet count < 150 x 10^9/L), but 5 neonates were excluded in the study as their medical files were either incomplete or missing. Of the remaining 15.6% (n=108) neonates with defined thrombocytopaenia whose medical files were available, thrombocytopaenia was almost equally distributed across the gender, 50.9% (n=55) for females, and 49.1% (n=53) for male infants; low birth weight infants (1500 - 2499g) were mostly affected, 41.7% (n=45). According to gestational age, infants born between 32 and 37 weeks of gestation were mostly affected than others, 50% (n=54). Thrombocytopaenia was seen more in neonates whose birth weight was appropriate for their gestational age, 59.3% (n=64), than in small for gestational age counterparts, 38.9% (n=42). Early onset thrombocytopaenia (≤ 72h of life) occurred in 55.6% (n=60), while 44.4% (n=48) were of late onset (>72h of life).

According to severity, neonatal thrombocytopaenia were predominantly mild, 51.9% (n=56). Twenty infants (18.5%) were diagnosed with sepsis based on a positive blood culture (bacterial and fungal), including 4 infants (20%) who demised. Of these, gram negative sepsis was the most common, 55% (n=11), with a mortality rate of 36.4% (n=4). Six infants (5.6%) had confirmed congenital abnormality with thrombocytopaenia. 3 of these infants (50%) were confirmed Trisomy 21. Sixteen (14.8%) of the infants diagnosed with thrombocytopaenia developed IVH. 43.8% (n=7) of these infants demised. NEC was seen in 15.7% (n=17), with 3 deaths (17.6%) occurring in this group. Of the 108 infants confirmed with thrombocytopaenia, only 3.7% (n=4) received a platelet transfusion in our unit, including 3 (75%) who demised. The most common maternal conditions were PET 29.6% (n=32), and PIH 21.3% (n=23). The study showed that chorioamnionitis was not a common maternal variable found in babies diagnosed with thrombocytopaenia in our unit, as only 0.93% (n=1) of this variable was identified during the study. Other main outcome measures identified were number of infants with thrombocytopenia discharged alive 87% (n=94) vs those who demised 13% (n=14). An equal gender distribution for mortality was seen with 50% mortality occurring in male and female infants.

CONCLUSION: The prevalence of neonatal thrombocytopaenia in NHCU of PTH between January 2017 and June 2017 was 15.6%. The thrombocytopaenia which were mostly mild (51.9%), occurred slightly more in female infants than their male counterparts (50.9% vs 49.1%). Of these, the low birth weight infants were mostly affected (41.7%). The study confirms thrombocytopaenia is a common problem in our unit, thus physicians caring for the infants must be aware of this in order to curb the morbidity and mortality burden thereof.

KEY WORDS:

Neonate, Neonatal thrombocytopaenia, Prevalence, Neonatal high care unit, Prematurity, Gestational age, Birth weight, Birth asphyxia, Sepsis, Necrotising enterocolitis.

1. CHAPTER 1: LITERATURE REVIEW

1.1. Introduction

Evaluation of a neonate with thrombocytopaenia is a challenge to neonatologists in both developed and developing countries. This is evidenced by lack of guidelines for the management of neonatal thrombocytopaenia (1,2). Developing a workable diagnostic strategy regarding the evaluation of these neonates with thrombocytopaenia is key towards prevention of death or neurological sequelae in the severely thrombocytopaenic neonates.

Whereas mild to moderate thrombocytopaenia (platelet count 51-149 x 10^9/L), can resolve spontaneously within the first ten days of life, requiring no clinical intervention, neonates with severe thrombocytopaenia (platelet count < 50 x 10^9/L) will require proper clinical work-ups and in some cases, multiple platelet transfusions (1,10).

This study aims to investigate and determine the prevalence of thrombocytopaenia in the neonates admitted to the neonatal high care unit of Pelonomi Tertiary Hospital in Bloemfontein, Free State province of South Africa, and also to describe the maternal/perinatal and neonatal characteristics found in neonates diagnosed with thrombocytopaenia in the unit.

1.2. Definition of neonatal thrombocytopaenia

Several studies in the past had similar findings with regards to the genesis of platelet production in the foetus. Some existing data have shown that production of platelets in foetuses can commence as early as five weeks after conception and that by the end of the second trimester life, foetuses actually have estimated normal postnatal platelet range of 150 - 450 x 10^9/L, which is the same range observed in older children and adults (1,13).

Thus, the definition of thrombocytopaenia is the same for preterm and term neonates, as it is in adults, which is a platelet count of < 150 x 10^9/L (1-17). Neonatal thrombocytopaenia can be classified based on the timing of presentation after birth into "early onset" (occurring ≤ 72 hours after delivery) and "late onset" (occurring >72 hours after delivery) (1-12). Based on severity, the thrombocytopaenia can be mild (platelet count of 101-149 x 10^9/L), moderate (platelet count of 51-100 x 10^9/L), severe (platelet count of 21-50 x 10^9/L), or very severe (platelet count of ≤20 x 10^9/L) (8,13).

1.3. Prevalence of neonatal thrombocytopaenia

Thrombocytopaenia is one of the most common haematological problems in newborns (2-5,10,12-14,17). Researchers differ as far as its prevalence in both healthy and sick neonates. In a healthy neonatal population, Eslami et al (2013) reported an occurrence of 1-2% (10), whereas a lower prevalence has been reported by other authors (7,12,16). A handful of authors have reported a higher incidence of 1-5% in healthy neonates (4,8,9,17). The prevalence was however reportedly much higher in sick neonates admitted to a neonatal intensive care unit (NICU), again, with considerable variation between the researchers. While some reviews reported a prevalence of 18-35% (2,10,14,15) in sick neonates, others reported a prevalence of 22-35% (1,4,8,9,13,17). In their cohort study of thrombocytopaenia in neonatal sepsis, Ree et al (2017) found an incidence of 49% of thrombocytopaenia in all neonates with proven culture positive sepsis admitted to a tertiary NICU (13). In the same study, the authors found that 20% of the neonates with confirmed sepsis had severe or very severe thrombocytopaenia (13). The reason for this significant variations in the prevalence and incidence of neonatal thrombocytopaenia reported among researchers may be due to the different populations of these studies (19).

Researchers also found that the prevalence varied depending on the gestational age (GA) and birth weights of the neonates, with the prevalence approaching 70% in the extremely low birth weight neonates (<1000g) (1,18–20). Studies have shown that besides GA and sepsis, high incidence of thrombocytopaenia can be found in neonates with other conditions. For instance, in their retrospective case control studies, Boutaybi et al (2014) found that the vast majority (80%) of neonates with perinatal asphyxia treated with therapeutic hypothermia developed thrombocytopaenia (9). The prevalence however, was reportedly low in some studies. In the study of Bolat et al (2012), the prevalence was 9.4% (18). In the same study Bolat et al (2012) found that 97 of the 208 neonates studied (46.6%) had mild thrombocytopaenia, 64 neonates (30.8%) had moderate thrombocytopaenia, 33 neonates (15.9%) had severe, and 14 neonates (6.7%) had very severe thrombocytopaenia (18). In contrast, Ayadi et al (2016) concluded that of the 808 neonates admitted to their NICU, mild thrombocytopaenia was found in 22.3%, moderate in 36.7%, and severe in 41% (5).

1.4. Causes of neonatal thrombocytopaenia

There are numerous identified causes of neonatal thrombocytopenia which are differentiated according to the time of presentation (early vs late) or mode of occurrence (congenital vs acquired).

1.4.1. Congenital causes

FNAIT occurs in approximately 1 in 1000 live births (19,20) and is the leading cause of early onset moderate or severe thrombocytopaenia in otherwise healthy appearing neonates (2,14,19,20). When the mother produces IgG antibodies against foetal antigens (alloantigens) inherited from the father, these allo-antibodies can cross the placenta and destroy the foetal platelets resulting in foetal and neonatal alloimmune thrombocytopaenia (FNAIT) (1,2,19,20). Also, a mother with immune thrombocytopaenic purpura (ITP) can produce autoantibodies which transplacentally destroy the foetal platelets resulting in immune thrombocytopaenia (1,2,20).

Congenitally acquired infections such as TORCH (toxoplasma gondii, rubella, cytomegalovirus and herpes simplex virus), enterovirus and human immunodeficiency virus (HIV), can all cause foetal and neonatal thrombocytopaenia (6,14).

Genetic disorders, e.g. trisomy 13, 18, and 21 can also cause thrombocytopaenia in the neonates (6).

1.4.2. Acquired causes

The most frequent acquired causes of early onset neonatal thrombocytopaenia are chronic foetal hypoxia, resulting from intrauterine growth restriction (IUGR), and maternal disorders such as maternal diabetes, pre-eclampsia/pregnancy-induced hypertension (PIH), or HELLP syndrome (1,2,6,8,10-12,14,17,18). The resulting thrombocytopaenia is usually mild and self-limiting (14) (Table 1.1). On the other hand, late onset thrombocytopaenia, usually severe, is almost always caused by sepsis or NEC (1,2,6,8,10-12,14,17,18) (Fig.1.1).

Table 1.1: Aetiologies of early-onset neonatal thrombocytopaenia (14).

Severity	Mild-to-moderate thrombocytopaenia, rarely severe	
Course	Slow, self-limiting	
Causes		
	Frequent	**Maternal/placental** • Pre-eclampsia/eclampsia, placental insufficiency, HELLP syndrome, hypertension, gestational diabetes • Rhesus haemolytic disease In case of severe thrombocytopaenia • Bacterial infection • Asphyxia • Congenital, mostly viral infections • Alloimmune thrombocytopaenia • Alloimmune hepatitis
	Rare	**Congenital** • Trisomy 13, 18, 21 • Thrombocytopaenia absent radii syndrome • Congenital amegakaryocytic thrombocytopaenia • Wiskott-Soulier syndrome • Bernard-Soulier syndrome • MYH9-associated diseases • Inborn errors of metabolism (Gaucher disease, methylmalonic acidaemia) • Kasabach Merrit, haemangioendothelioma

HELLP: haemolysis, elevated liver enzymes, low platelet count.

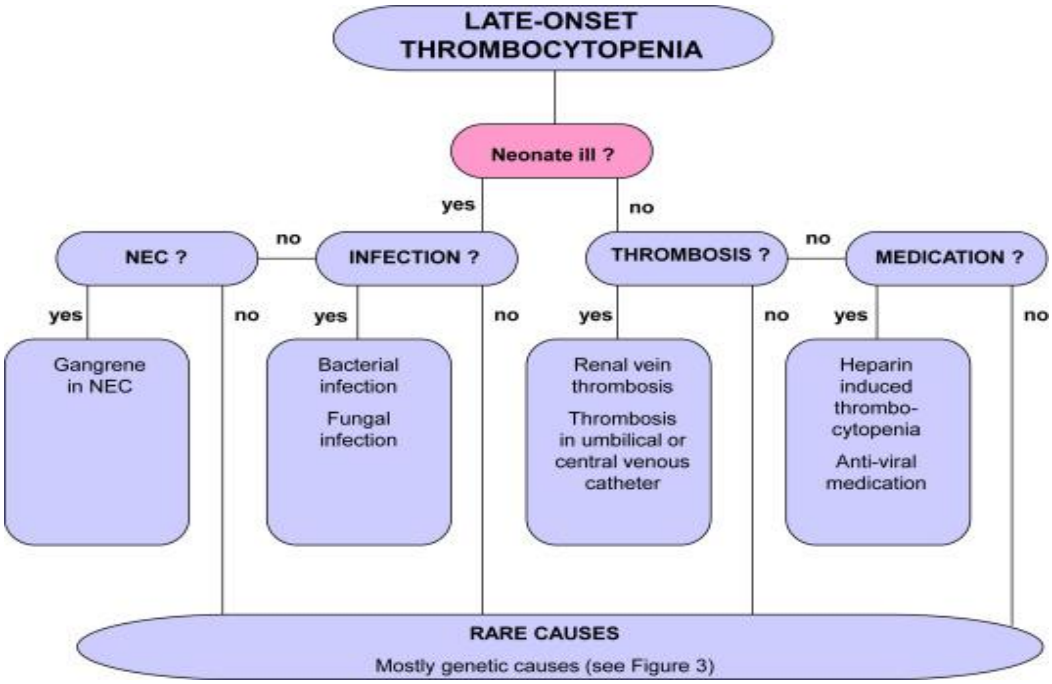


Figure 1.1: Algorithm for neonatal late-onset thrombocytopaenia (>72h postnatal age) when considering the differential diagnosis. NEC, necrotizing enterocolitis (14).

1.5. Mechanisms of neonatal thrombocytopaenia

Three different kinetic mechanisms responsible for thrombocytopaenia in neonates as well as in adults have been described. These include (12,18):

(i) Decreased platelet production (e.g. thrombocytopaenia seen in babies born to mothers with severe placental insufficiency);
(ii) Increased platelet destruction (e.g. sepsis or NEC, ITP, auto-immune causes);
(iii) Platelet sequestration (mostly secondary to hypersplenism);

Or a combination of these processes, which in some reports is identified as the number four mechanism.

1.6. Thrombocytopaenia and associated risk factors in neonates

The risk factors associated with the development of thrombocytopaenia in neonates can be divided into maternal and neonatal factors. Many authors have linked multiple neonatal and maternal conditions as causative factors of thrombocytopaenia in the neonates admitted to NICU.

1.6.1. Maternal and perinatal risk factors

In their retrospective one-year period of study of 350 neonates admitted to their NICU, of which 100 (28.6%) had thrombocytopaenia, Eslami et al (2013) analysed the contributory effects of both maternal and neonatal variables and concluded that the significant maternal risk factors that led to thrombocytopaenia in the neonates studied were pregnancy induced hypertension (PIH), gestational diabetes mellitus (GDM), eclampsia and autoimmune disease such as immune thrombocytopaenic purpura (ITP) (10). The results showed that the most common maternal risk factor was PIH (Table 1.2).

Table 1.2: Incidence maternal risk factors in thrombocytopaenic neonates (10).

Maternal Risk Factor	Frequency	
	N	%
PIH	13	46.4
GDM	9	32.1
GDM+PIH	3	10.7
Eclampsia	2	7.1
ITP	1	3.6

GDM: gestational diabetes mellitus; ITP: immune thrombocytopaenic purpura; PIH: pregnancy induced hypertension.

Similar findings were made by other researchers in their various studies (4,5). In contrast to most studies, Goyal et al (2017), did not find any maternal risk factors in their study for neonatal thrombocytopaenia (7). In another study by Saini et al (2017), premature rupture of membranes (PROM) (20.5%), and antepartum haemorrhage (APH) (17.87%) were the leading maternal causative factors for neonatal thrombocytopaenia, while PIH contributed to only 4.94% (3).

1.6.2. Neonatal risk factors

Several studies have shown significant association between multiple neonatal variables like sepsis, birth asphyxia, and low birth weight (LBW), intrauterine growth restriction (IUGR), small-for-gestational age (SGA), ABO incompatibility, and necrotising enterocolitis (NEC) and neonatal thrombocytopaenia. Eslami et al (2013) found out that the most common neonatal risk factor for thrombocytopaenia was sepsis (31.9%), followed by IUGR (20.8%) (Table 1.3) (10).

Table 1.3: Incidence neonatal risk factors in thrombocytopaenic neonates (10).

Neonatal Risk Factor	Frequency	
	N	%
Sepsis	23	31.9
IUGR	15	20.8
Asphyxia	10	13.9
Asphyxia + IUGR	5	6.9
Sepsis + IUGR	2	2.8
Asphyxia + Sepsis	1	1.4
NEC	2	2.8
ABO	3	4.2
Other	11	15.3
Sum	72	100

ABO: blood group system A, B, O; IUGR: intrauterine growth restriction; NEC: necrotizing enterocolitis.

These findings were similar to other studies (4,5,7,13). Some researchers however differ in their observations concerning causal relationship between these neonatal conditions and thrombocytopaenia. Fustolo-Gunnik et al (2016) linked early-onset thrombocytopaenia to SGA neonates (8). In the same comparative study, the authors found that SGA neonates were at increased risk of early-onset thrombocytopaenia compared to their appropriate gestational age (AGA) counter parts. Boutaybi et al (2014) did a retrospective case control study on neonates with perinatal asphyxia treated with therapeutic hypothermia, and found that 80% of the study population had thrombocytopaenia (9). In a different study by the same authors, perinatal asphyxia was found to be a risk factor in 51% of cases for early-onset neonatal thrombocytopaenia (17).

LBW, poor Apgar scores, prematurity and birth asphyxia were the causative factors found by Saini et al (2017) in their cross-sectional study at a tertiary care unit (3).

1.7. Diagnostic approach to neonatal thrombocytopaenia

1.7.1. Clinical history

In every neonate with suspected thrombocytopaenia, literature has shown that most of the possible causes can be determined by the clinical history and presentation. Thorough neonatal history (gestational age, birth weight, timing of onset) as well as maternal history including present and past pregnancies, and family history of hereditary thrombocytopaenia, are therefore advisable (21,22).

1.7.2. Laboratory investigations

Full blood count (FBC), including peripheral blood smears, can be done to ascertain the platelet count and to distinguish between macro-and micro-thrombocytopaenia respectively (22). The neonates should also be screened for any suspected specific disease including sepsis. The data collected from the clinical history and laboratory investigations can then be incorporated into a diagnostic algorithm for preterm (Fig. 1.2) and term (Fig. 1.3) neonates below (20).

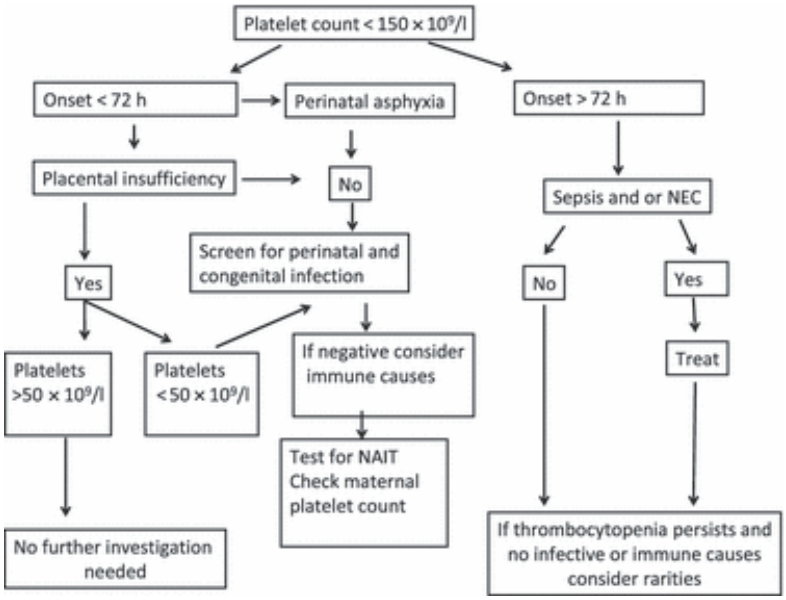


Figure 1.2: Diagnostic algorithm for investigation of preterm neonates with thrombocytopaenia. NEC, necrotizing enterocolitis; NAIT, neonatal alloimmune thrombocytopaenia (20).

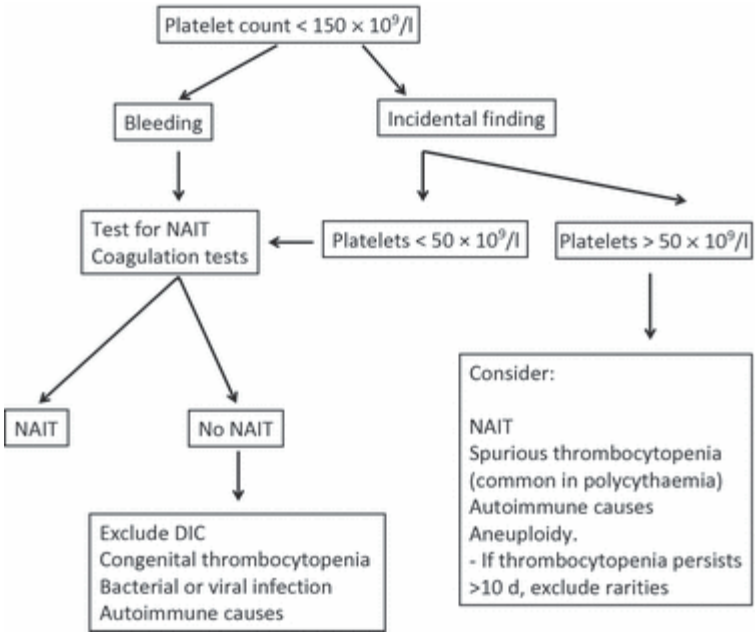


Figure 1.3: Diagnostic algorithm for investigation of term neonates with thrombocytopaenia. NAIT, neonatal alloimmune thrombocytopaenia; DIC, disseminated intravascular coagulation (20).

1.8. Management of thrombocytopaenia in the neonates

Besides supportive measures, the only management of thrombocytopaenia is platelet transfusion (6). There is a widespread agreement among researchers regarding the paucity of evidence-based practice in neonatal thrombocytopaenia and platelet transfusion thresholds in neonates admitted to NICU (1,2,6,14-16). This has created a wide variation in practice among neonatologists. For instance, prophylactic platelet transfusions are being given liberally at higher platelet thresholds in the USA and Canada compared to Europe (1,14-16).

1.8.1. Threshold for platelet transfusion

There is a great deal of variability regarding the platelet count below which a neonate with thrombocytopaenia should be transfused, especially in light of lack of evidence-based recommendations among neonatologists worldwide (1,2,14-16,21,22). Hence, decisions whether or not to transfuse are based on expert opinions in any given institution (1,2). A reasonably safe threshold for platelet transfusion for most neonates have been reported by various authors to be platelet count of 20 x 10^9/L (6). It is however, recommended that prophylactic platelets be given to all neonates, term or preterm, with a confirmed platelet count <20 x 10^9/L, to stable preterm infants if the count falls below 30 × 10^9/L and to all with a birth weight of <1,000 g if the platelets are <50 × 10^9/L during the 1st week (1,6).

Also, threshold of 50 × 10^9/L is commonly used for infants who are clinically unstable, have had a previous major bleed or have other recognised risk factors (1,6).

1.8.2. Platelet transfusion guidelines

The fact that no data exist yet which show whether or not liberal vs more restrictive platelet transfusion strategy has any influence on the outcome of severe thrombocytopaenia (1), is more confounding than helpful. Internationally, neonatologists are awaiting the outcome of the large multicentre randomised trial currently being conducted in several European countries by The Platelets for Neonatal Transfusion- study 2 (PlaNeT-2). This study compares liberal vs restrictive prophylactic platelet transfusion strategies (50 vs 20 x 10^9/L, respectively) in preterm neonates with thrombocytopaenia (14,16). The initiative, will no doubt be of great benefits if and when evidence-based standardised guidelines are formulated following this study. Until then, decision whether or not to transfuse is based on expert opinion (1). Reports have shown that whereas practices in UK and other European countries favour restrictive platelet transfusion strategy, US and Canadian neonatologists generally favour more liberal transfusion thresholds (1,14-16).

1.8.3. Guidelines in our local practice

There are no published existing platelet transfusion guidelines in the Free State Province. The Standard Treatment Guidelines and Essential Medicines List for South Africa, Hospital Levels Paediatrics (EDL) (2017) do not include much on neonatal thrombocytopaenia and no information is included regarding transfusion guidelines. In our local practice at Pelonomi Tertiary Hospital, the decision whether or not to transfuse neonates with severe thrombocytopaenia with platelets is at the discretion of the consultant on duty. This decision is usually based on the only available guidelines in our area (Table 1.4) (23), which are recommendations based on the Cape Town practices. According to these guidelines, a platelet count <30 x 10^9/L should form the transfusion threshold even if the neonate is asymptomatic (not bleeding) or not. This means that we may consider *prophylactic* platelets transfusion to all neonates, term or preterm, with a confirmed platelet count of <30 x 10^9/L, and *therapeutically* to neonates who are bleeding or with suspected or confirmed neonatal allo-immune thrombocytopaenia (NAITP) [Table 1.4].

Table 1.4: Guidelines for platelet transfusion thresholds for neonates (23).

Platelet count (x 10^9/L)	Non-bleeding neonate	Bleeding neonate	*NAITP (proven or suspected)
<30	Consider transfusion in all patients	Transfuse	Transfuse (with **HPA compatible platelets)
30-49	Do not transfuse if clinically stable. Consider transfusion if: • < 1000g and < 1 week of age • Hypotension requiring inotropic support • Previous major bleeding tendency (e.g. Grade 3-4 IVH) • Current minor bleeding (e.g. petechiae, puncture site oozing) • Current coagulopathy • Respiratory disease requiring FiO2 > 40% or MAP > 9cm • Seizures within the last 72 hours • Requires exchange transfusion • Pre-surgery (within 24 hours) • Post-surgery (within 5 days)	Transfuse	Transfuse (with HPA compatible platelets)
50-99	Do not transfuse	Transfuse	Transfuse (with HPA compatible platelets if major bleeding present)
>99	Do not transfuse	Do not transfuse	Do not transfuse

* NAITP: neonatal alloimmune thrombocytopaenia
** HPA: human platelet antigen.

With platelet counts 30-49 x 10^9/L, our guidelines advise not to transfuse if the neonate is clinically stable, but to transfuse if the following conditions apply: bleeding, has suspected or confirmed NAITP, < 1000g and < 1 week of age, hypotension requiring inotropic support, previous major bleeding tendency (e.g. Grade 3-4 IVH), current minor bleeding (e.g. petechiae, puncture site oozing), current coagulopathy, respiratory disease requiring FiO2 > 40% or MAP > 9cm, seizures within the last 72 hours, requires exchange transfusion, pre-surgery (within 24 hours), or post-surgery (within 5 days). If the neonate's platelet count is 50-99 x 10^9/L, transfusion is indicated only if the neonate is symptomatic with bleeding or has suspected or proven NAITP. However, with a platelet count >99 x 10^9/L, we do not transfuse despite the clinical condition of the neonate (Table 1.4) (23).

1.9. Possible outcomes of neonatal thrombocytopaenia

1.9.1. Neonatal thrombocytopaenia and risk of bleeding

Whereas thrombocytopaenia can be a risk factor for haemorrhage, the majority of neonates with thrombocytopaenia do not develop major haemorrhage (11). Existing data suggest that there is a poor correlation between severity of thrombocytopenia and clinically significant bleeding, meaning that factors other than the platelet count determine the bleeding risk (2). Studies have shown that the risk of bleeding in neonates with thrombocytopaenia is highest in the preterm neonates with FNAIT, sepsis and NEC in the first week of life (2,12,22). Bleeding symptoms vary according to the individual neonate, and most of them are mild (22). There can be cutaneous bleed, intracranial, pulmonary or gastrointestinal bleedings. In their retrospective analysis of 134 neonates with thrombocytopaenia, Ulusoy et al (2013) found that 11% had pathological haemorrhage (intracranial haemorrhage 50%, pulmonary haemorrhage 32%, and gastrointestinal haemorrhage 18% (12). These haemorrhages were significantly more common in thrombocytopaenic neonates who were premature and with sepsis (12).

1.9.2. Neonatal thrombocytopaenia and mortality rates

Studies have shown high mortality rates in neonates with severe thrombocytopaenia who received platelet transfusions (12,18). However, it is unclear whether the high mortality is a result of underlying conditions causing severe thrombocytopaenia, or a direct effect of platelet transfusion itself (12). When compared with neonates without thrombocytopaenia, mortality rates have been found to be high in neonates with severe or very severe thrombocytopaenia (18). The mortality is even higher in thrombocytopaenic neonates with confirmed gram negative bacterial or fungal sepsis (11).

1.10. Relationship between severity of thrombocytopaenia and risk of bleeding and mortality

There is poor correlation between the severity of thrombocytopaenia and risk of bleeding and or mortality in neonates with thrombocytopaenia (2,12,15).

1.11. Problem statement

Besides phlebotomy-induced anaemia, thrombocytopaenia is the commonest haematological abnormality encountered in neonates admitted to NICU. This condition is more prevalent in the very low birth weight and sick premature neonates who constitute the highest population in our neonatal high care unit. Hence, neonatal thrombocytopaenia is a problem in our local practice. It is therefore pertinent to have a working diagnostic approach and proper management guidelines when dealing with these sick neonates.

Literature studies have highlighted the problem of lack of evidence-based data locally and internationally regarding management guidelines of neonatal thrombocytopaenia and the challenges faced by the clinicians all over the world.

1.12. Future Research

Even though only infants admitted to the unit within 72 hours of birth were included in the study, some of them had initial FBCs taken only after 3 days of life, it cannot therefore be proven when the thrombocytopaenia occurred, making classification according to timing of thrombocytopaenia (early vs late) difficult, if not impossible. A further study looking at serial FBCs taken timeously is therefore required to prove the timing of thrombocytopaenia. Also, the study described some neonatal and maternal/perinatal conditions found in the neonates diagnosed with thrombocytopaenia, but since this is not a cross-sectional or case-controlled study, the causality could not be measured, therefore, a further study designed accordingly is required to elucidate the causal relationship between these factors and the risk of development and severity of thrombocytopaenia in neonates.

2. CHAPTER 2: ARTICLE MANUSCRIPT

2.1. ABSTRACT

BACKGROUND: Thrombocytopaenia is the most common haematological abnormality, besides anaemia, found in the neonates admitted to a neonatal intensive care unit. Even though most cases are mild or moderate, requiring minimal or no intervention, and resolving spontaneously within the first week of life; a few infants, especially the very sick ones or the extremely low birth weights (<1000g), may present with severe and in some cases persistent thrombocytopaenia, which may require intervention such as platelet transfusion.

OBJECTIVES: The primary objective of this study was to determine the prevalence of thrombocytopaenia in neonates admitted to the neonatal high care unit (NHCU) of Pelonomi Tertiary Hospital (PTH).

METHODS: The study was a retrospective descriptive study conducted on 694 infants admitted to the NHCU of PTH between January 2017 and June 2017. Data included were neonatal demographic information, information regarding the clinical data of neonate and the mother obtained from the neonatal admission book used routinely at PTH by the admitting medical doctor. Infants with thrombocytopaenia were identified using the National Health Laboratory Service web results viewer. The data regarding the rate of performance of platelet count per patient in the cohort group was not collected as the primary objective was to determine whether or not thrombocytopaenia was present in the samples taken within the 72 hours of birth. Also, the common reason for doing blood investigations in the cohort group was not specified during the data collection because this attribute was not documented in the patients' records. The data analysis was performed using the SAS System version 9.4.

RESULTS: During the study period, a total of 694 neonates were admitted to the NHCU of PTH. Of these, 16.3% (n=113) infants were diagnosed with thrombocytopaenia (platelet count < 150 x 10^9/L), but 5 neonates were excluded in the study as their medical files were either incomplete or missing. Of the remaining 15.6% (n=108) neonates with defined thrombocytopaenia whose medical files were available, thrombocytopaenia was almost equally distributed across the gender, 50.9% (n=55) for females, and 49.1% (n=53) for male infants; low birth weight infants (1500 - 2499g) were mostly affected, 41.7% (n=45). According to gestational age, infants born between 32 and 37 weeks of gestation were mostly affected than others, 50% (n=54). Thrombocytopaenia was seen more in neonates whose birth weight was appropriate for their gestational age, 59.3%

(n=64), than in small for gestational age counterparts, 38.9% (n=42). Early onset thrombocytopaenia (≤ 72h of life) occurred in 55.6% (n=60), while 44.4% (n=48) were of late onset (>72h of life). According to severity, neonatal thrombocytopaenia were predominantly mild, 51.9% (n=56). Twenty infants (18.5%) were diagnosed with sepsis based on a positive blood culture (bacterial and fungal), including 4 infants (20%) who demised. Of these, gram negative sepsis was the most common, 55% (n=11), with a mortality rate of 36.4% (n=4). Six infants (5.6%) had confirmed congenital abnormality with thrombocytopaenia. 3 of these infants (50%) were confirmed Trisomy 21. Sixteen (14.8%) of the infants diagnosed with thrombocytopaenia developed IVH. 43.8% (n=7) of these infants demised. NEC was seen in 15.7% (n=17), with 3 deaths (17.6%) occurring in this group. Of the 108 infants confirmed with thrombocytopaenia, only 3.7% (n=4) received a platelet transfusion in our unit, including 3 (75%) who demised. The most common maternal conditions were PET 29.6% (n=32), and PIH 21.3% (n=23). The study showed that chorioamnionitis was not a common maternal variable found in babies diagnosed with thrombocytopaenia in our unit, as only 0.93% (n=1) of this variable was identified during the study. Other main outcome measures identified were number of infants with thrombocytopenia discharged alive 87% (n=94) vs those who demised 13% (n=14). An equal gender distribution for mortality was seen with 50% mortality occurring in male and female infants.

CONCLUSION: The prevalence of neonatal thrombocytopaenia in NHCU of PTH between January 2017 and June 2017 was 15.6%. The thrombocytopaenia which were mostly mild (51.9%), occurred slightly more in female infants than their male counterparts (50.9% vs 49.1%). Of these, the low birth weight infants were mostly affected (41.7%). The study confirms thrombocytopaenia is a common problem in our unit, thus physicians caring for the infants must be aware of this in order to curb the morbidity and mortality burden thereof.

2.2. INTRODUCTION

Thrombocytopaenia is the most common haematological abnormality, besides anaemia, found in infants admitted in a neonatal intensive care unit (2). Even though most cases are mild or moderate, requiring minimal or no intervention, and resolving spontaneously within the first week of life; a few infants, especially the very sick ones or the extremely low birth weights (<1000g), may present with severe and in some cases persistent thrombocytopaenia, which may require intervention such as platelet transfusion (1-12).

Causes of thrombocytopaenia in neonates are multifactorial. Maternal conditions, especially those resulting in placental insufficiency, as well as foetal factors have all been implicated.

Early onset thrombocytopaenia (occurring ≤72 hours of life) usually results from maternal conditions such as ITP, gestational diabetes, drug use or from complications of pregnancy such as intrauterine growth restriction, HELLP syndrome as well as chronic foetal hypoxia. Conversely, late onset thrombocytopaenia (occurring >72 hours of life) is almost always a result of sepsis or necrotizing enterocolitis (1–12). Thrombocytopaenia occurring in these conditions are usually severe in nature warranting multiple platelet transfusions (1,10). Complications posed by severe thrombocytopaenia in preterm infants admitted in a neonatal intensive care unit make up part of the global burden of neonatal morbidity and mortality faced by neonatologists.

Currently there is significant disagreement among practitioners with regards to the platelet count which a newborn infant with thrombocytopaenia should be transfused (2), especially in light of lack of evidence-based recommendations among neonatologists worldwide with regards to transfusion thresholds. Decisions whether or not to transfuse are basically expert opinions in any given institution (1,2,14). Despite this dilemma however, appropriate diagnostic and therapeutic management guidelines are necessary to prevent death or neurological sequelae in the severely thrombocytopaenic infants.

The aim of this study was to determine the prevalence of neonatal thrombocytopaenia at Pelonomi Tertiary Hospital over a six-month period. No similar study had been done in the past in our area, hence, until this study, the seriousness and extent of neonatal thrombocytopaenia in our practice was basically unknown. This study therefore became necessary in order to look at the gravity and scope of this problem in our practice and also to look at how we manage infants with thrombocytopaenia especially with regards to our attitude towards platelet transfusion in babies with thrombocytopaenia and their ultimate outcome in the unit.

2.3. METHODS

The main outcome measure was to determine the prevalence of thrombocytopaenia and also to describe the maternal and neonatal characteristics found in infants with thrombocytopaenia in our unit. The outcomes of neonates diagnosed with thrombocytopaenia with or without platelet transfusion were also investigated.

The study was a retrospective descriptive study conducted on 694 infants admitted to the neonatal high care unit of Pelonomi Tertiary Hospital (PTH) between January 2017 and June 2017. All neonates born and admitted during the study period were screened for inclusion, therefore a sampling method was not required. Participants were identified using the admission registers as well as the National Health Laboratory Service (NHLS) trackcare web results viewer. Patients with defined thrombocytopaenia were identified and their medical files were obtained for data collection purposes. Only neonates admitted to the neonatal high care unit within 72 hours of birth were included in the study, because it would have been difficult to determine when they became thrombocytopenic after birth if admitted after 72 hours of life, and classification into early or late onset would have been impossible. Participants were excluded if their medical files were missing or incomplete or if their laboratory results didn't include platelet counts. The data regarding the rate of performance of platelet count per patient in the cohort group was not collected as the primary objective was to determine whether or not thrombocytopaenia was present in the samples taken within the 72 hours of birth. Also, the common reason for doing blood investigations in the cohort group was not specified during the data collection because this attribute was not documented in the patients' records.

Data included were neonatal demographic information (gender, gestational age, birth weight), as well as date and time of birth and admission. This information, as well as the information regarding the clinical data of neonate (e.g. congenital abnormality, birth asphyxia, Apgar scores at 5min, blood gas base excess, sepsis, platelet values, timing of onset of thrombocytopaenia, IVH, NEC, and outcome) and the maternal characteristics (including history of APH, PIH, PET/Eclampsia, GDM, PROM and chorioamnionitis) were obtained from the neonatal admission book used routinely at PTH by the admitting medical doctor. The data analysis was performed using the SAS System version 9.4.

2.4. RESULTS

During the study period, a total of 694 neonates were admitted to the NHCU of PTH. Of these, 16.3% (n=113) infants were diagnosed with thrombocytopaenia (platelet count < 150 x 10^9/L), but 5 neonates were excluded in the study as their medical files were either incomplete or missing. Of the remaining 15.6% (n=108) neonates with defined thrombocytopaenia whose medical files were available, thrombocytopaenia was almost equally distributed across the gender, 50.9% (n=55) for females, and 49.1% (n=53) for male infants. Low birth weight infants (1500 - 2499g)

were mostly affected, 41.7% (n=45), followed by very low birth weight infants, 27.8% (n=30), (Table 2.1).

Table 2.1: Distribution of thrombocytopaenia in the unit across birth weights.

Birth weight category	Frequency	Percent (%)
NBW(≥2500g)	18	16.7
LBW (1500-2499g)	45	41.7
VLBW (1000-1499g)	30	27.8
ELBW (≤999g)	15	13.9

NBW: normal birth weight; LBW: low birth weight; VLBW: very low birth weight; ELBW: extremely low birth weight.

According to gestational age, infants born between 32 and 37 weeks of gestation were mostly affected than others, 50% (n=54). The extremely premature babies, born at less than 28 weeks of gestation were the least affected with an incidence of 4.63% (n=5).

Contrary to the robust evidence which suggest otherwise, in this study, thrombocytopaenia was seen more in neonates whose birth weights were appropriate for their gestational age, 59.3% (n=64), than their smaller for gestational age counterparts, 38.9% (n=42), the remaining 1.9% (n=2) were found in neonates classified as large for gestational age. Early onset thrombocytopaenia (≤ 72h of life) occurred in 55.6% (n=60) (Table 2.2), whereas 44.4% (n=48) were of late onset (>72h of life).

Table 2.2: Classification of thrombocytopaenia according to the timing of onset.

Timing of onset:	**Frequency:**	**Percent (%):**
Early onset (≤72h of life)	60	55.6
Late onset (>72 of life)	48	44.4

According to severity (Table 2.3), neonatal thrombocytopaenia were predominantly mild, 51.9% (n=56), followed by moderate, 27.8% (n=30), then severe, 14.8% (n=16), and very severe, 5.6% (n=6).

Table 2.3: Classification of thrombocytopaenia in the unit according to severity.

Severity:	Frequency:	Percent (%):
Mild (platelet count of 101-149 x 10^9/L)	56	51.9
Moderate (platelet count of 51-100 x 10^9/L)	30	27.8
Severe (platelet count of 21-50 x 10^9/L)	16	14.8
Very severe (platelet count of ≤20 x 10^9/L)	6	5.6

Twenty infants (18.5%) were diagnosed with confirmed blood culture bacterial and fungal sepsis, see Table 2.4 below, including 4 infants (20%) who demised.

Table 2.4: Thrombocytopaenia in infants with confirmed sepsis in the unit through blood culture.

Organisms:	Frequency	Percent (%)
Single cultures:		
a) Acinetobacter baumanni	7	35
b) Candida albican	1	5
c) Candida parapsilosis	2	10
d) Clostridium monocytogenes	1	5
e) Corynebacterium species	1	5
f) Enterococcus faecalis	1	5
g) Klebsiella pneumonia	4	20
Mixed cultures:		
a) A. baumanni + S. aureus + GBS	1	5
b) A. baumanni + Yeast	1	5
c) A. baumanni + E. cloacae	1	5
Confirmed sepsis:	20	18.5
No sepsis:	88	81.5

A. baumanni: Acinetobacter baumanni; E. cloacae: Enterobacter cloacae; GBS: group B streptococcus; S. aureus: Staphylococcus aureus.

Of these, gram negative sepsis was predominant, 55% (n=11) (Table 2.4) with a mortality rate of 36.4% (n=4) in this group. Overview of the severity of thrombocytopaenia in the confirmed septic neonates are shown graphically in Table 2.5 below, with majority (35%) having moderate thrombocytopaenia (n=7). Only 15% (n=3) of the septic neonates were severely thrombocytopaenic, whereas 25% (n=5) had platelet counts ≤20 x 10^9/L (very severe thrombocytopaenia).

Table 2.5: Severity of thrombocytopaenia in confirmed sepsis (n=20).

Severity	Frequency	Percent (%)
Mild	5	25
Moderate	7	35
Severe	3	15
Very severe	5	25
Confirmed sepsis:	**20**	**100**

Of the 108 infants admitted to the NHCU of PTH who were studied, 9 of them (8.3%) had congenital abnormalities with thrombocytopaenia (Table 2.6). Six of these infants' congenital abnormalities were confirmed, including 3 neonates with confirmed Trisomy 21. Equally, 3 babies were phenotypically described as being dysmorphic without a further diagnosis available (Table 2.6).

Table 2.6: Distribution of thrombocytopaenia in the unit across infants with congenital abnormalities.

Congenital abnormality	Frequency	Percent (%)
Trisomy 21	3	2.8
Trisomy 13	1	0.9
Turner syndrome	1	0.9
Silver Russell syndrome	1	0.9
Dysmorphism (not specified)	3	2.8
Those without congenital abnormality	99	91.7

Sixteen (14.8%) of the infants diagnosed with thrombocytopaenia developed IVH, of these, more than half had IVH grade II, 56.3% (n=9), while 31.3% (n=5) were not graded (Table 2.7) below. Seven of the neonates diagnosed with IVH in the unit (43.8%) had moderate thrombocytopaenia, with majority, 62.5% (n=10) being of late onset. Only 1 (6.3%) and 2 (12.5%) had severe and very severe thrombocytopaenia respectively. Of the 16 thrombocytopaenic neonates complicated with IVH, 43.8% (n=7) mortality was recorded in the unit. Of the IVH group, only 3 (18.8%) received platelet transfusion in the unit, with poor outcome, as all demised (Table 2.7). But overall, of the 108 infants studied, only 3.7% (n=4) received platelet transfusion in our unit.

Comparably, NEC was seen in 15.7% (n=17), with 3 deaths (17.6%) occurring in this group. Five (29.4%) of the NEC infants were confirmed septic at the time of diagnosis with thrombocytopaenia, majority being gram negative or fungal sepsis (Table 2.8) below.

Table 2.7: Distribution of severity of thrombocytopaenia in neonates diagnosed with IVH, management received in terms of platelet transfusion, and their ultimate outcome in the unit (n=16).

	Frequency:	Percent (%):
Severity of thrombocytopenia:		
a) mild	6	37.5
b) moderate	7	43.8
a) severe	1	6.3
b) very severe	2	12.5
Timing of onset of thrombocytopenia:		
a) early onset	6	37.5
b) late onset	10	62.5
Grade of IVH:		
a) grade I	2	12.5
b) grade II	9	56.3
c) grade III-IV	0	00.0
d) not graded	5	31.3
Outcome:		
a) discharged	9	56.3
b) demised	7	43.8
Mortality group (n=7):		
1) Severity of thrombocytopaenia:		
a) mild	3	42.9
b) moderate	3	42.9
c) severe	0	00.0
d) very severe	1	14.3
2) Platelet transfusion received:		
a) yes	3	42.9
b) no	4	57.1

IVH: intraventricular haemorrhage.

Table 2.8: Distribution of severity of thrombocytopaenia in neonates diagnosed with NEC, management received in terms of platelet transfusion, and their ultimate outcome in the unit (n=17).

	Frequency:	Percent (%):
Severity of thrombocytopenia:		
a) mild	6	35.3
b) moderate	7	41.2
c) severe	3	17.6
d) very severe	1	5.9
Timing of onset of thrombocytopenia:		
a) early onset	0	00.0
b) late onset	17	100.0
Grade of NEC:		
a) grade I (a/b)	3/1	17.6/5.9
b) grade II (a/b)	4/0	23.5/00.0
c) grade III (a/b)	0/0	00.0/00.0
d) not graded	9	52.9
Sepsis at time of diagnosis with NEC:		
a) yes (1=CNS; 2=GNS; 1=FS; 1=GNS+FS)	5	29.4
b) no	12	70.6
Outcome:		
a) discharged	14	82.4
b) demised	3	17.6
Mortality group (n=3):		
1) Severity of thrombocytopaenia:		
a) mild	1	33.3
b) moderate	1	33.3
c) severe	1	33.3
d) very severe	0	00.0
2) Platelet transfusion received:		
a) yes	0	00.0
b) no	3	100.0

CNS: coagulase negative staphylococcus; GNS: gram negative sepsis; NEC: necrotizing enterocolitis; FS: fungal sepsis.

The overall mortality of the thrombocytopaenic neonates in the unit was 13% (n=14). Table 2.9 below describes the distribution of mortality across each category in the unit. The study showed that the infants whose deaths were related to HIE with low platelets as a confounding factor, had the highest mortality rate of 39.3% (n=11).

Table 2.9: Distribution of mortality rate across each category in the unit (n=14).

Category:	Frequency:	Percent (%):
Confirmed sepsis	4	14.3
Infants with confirmed IVH	7	25
Infants with confirmed NEC	3	10.7
Infants with birth asphyxia	11	39.3
Infants who received platelet transfusion	3	10.7

NEC: necrotizing enterocolitis; IVH: intraventricular haemorrhage.

The most common maternal characteristics found in the neonates with thrombocytopaenia in the unit, described as risk factors in the literature, were PET 29.6% (n=32), and PIH 21.3% (n=23) (Table 2.10). The study showed that chorioamnionitis was not a common maternal variable found in babies diagnosed with thrombocytopaenia in our unit, as only 0.93% (n=1) of this variable was identified during the study (Table 2.10).

Table 2.10: Distribution of thrombocytopaenia in the unit across maternal/perinatal conditions.

Maternal/perinatal conditions:	Frequency:	Percent (%):
APH	9	8.3
CA	1	0.9
GDM	1	0.9
HELLP	4	3.7
PET	32	29.6
PIH	23	21.3
PROM	6	5.6
NO MATERNAL/PERINATAL CONDITION	32	29.6

APH: antepartum haemorrhage; CA: chorioamnionitis; GDM: gestational diabetes mellitus; HELLP: haemolysis, elevated liver enzymes, low platelet count; PET: pre-eclamptic toxaemia; PIH: pregnancy induced hypertension; PROM: premature rupture of the membrane.

2.5. DISCUSSION

This study confirms that neonatal thrombocytopaenia is a common problem in our neonatal high care unit at PTH. In this study, thrombocytopaenia was diagnosed in 108 of the 694 infants admitted to NHCU of PTH between January 2017 and June 2017. The findings of the study showed that the prevalence of neonatal thrombocytopaenia at PTH was 15.6%, which is lower than the rates reported in the literature (2,10,14,15). As reported by Tiller et al (2017), the incidence of thrombocytopaenia in neonates varies significantly, depending on the population studied (19). In the NHCU of PTH, we admit neonates of different disease profiles and birth weights, this may therefore explain the reason for the lower prevalence reported in this study. Thrombocytopaenia was almost equally distributed across the gender, 50.9% (n=55) for females, and 49.1% (n=53) for male infants. This is in agreement with the findings made by Eslami et al (2013) who did not find any significant gender differences in the incidence of thrombocytopaenia (10). This finding is, however, in contrast to the views of Ulusoy et al (2013) who found that thrombocytopaenia was more common in males (64%) than in females (12).

According to severity, and in agreement with existing data (3,17,18), neonatal thrombocytopaenia found in our unit were predominantly mild, 51.9% (n=56). A graphic representation of these data is shown in Table 2.3 above.

The study also showed that 55.6% (n=60) of the thrombocytopaenia were of early onset, whereas 44.4% (n=48) were of late onset (Table 2.2). Several studies reviewed in the literature showed that the underlying cause of neonatal thrombocytopaenia can be predicted with onset of the timing of thrombocytopaenia after birth, with most findings reported in these studies suggesting that the most frequent cause of early onset thrombocytopaenia is associated with chronic foetal hypoxia, resulting from IUGR and maternal disorders such as PIH, PET, HELLP syndrome, or GDM (1,2,6,8,10-12,14,17,18). On the other hand, according to the same studies, late onset thrombocytopaenia is almost always caused by sepsis or NEC. With the exception of IUGR, not described in this study, the majority of early onset thrombocytopaenia seen in our unit, 65.8% (n=50) was found in neonates born to mothers with APH, PIH, PET/Eclampsia, HELLP syndrome, PROM, GDM and chorioamnionitis (Table 2.10), while 78.4% (n=29) of the late onset thrombocytopaenia described were found in neonates confirmed with sepsis and NEC. Thus, these findings were in accord with those reviewed in the literature.

Also, in concordance with most studies (7,8,14), the early onset thrombocytopaenia found in our unit was mild to moderate in severity (61.3% vs 27.4%). Contrary to evidence which suggest otherwise (7,8), in this study, thrombocytopaenia was seen more in neonates whose birth weights were appropriate for their gestational age, 59.3% (n=64), than in small for gestational age counterparts, 38.9% (n=42), the remaining 1.9% (n=2) was found in neonates classified as large for gestational age. Low birth weight infants (1500 - 2499g) were mostly affected (Table 2.1), 41.7% (n=45), followed by very low birth weight infants, 27.8% (n=30). According to the findings by Saini et al (2017) in their cross-sectional observational study (3), LBW (68.06%) was the major risk factor of neonatal thrombocytopaenia in their unit. Conversely, according to majority of data reviewed, the prevalence of thrombocytopaenia in neonates admitted to NICU have been found to be inversely related to the gestational age and birth weights of the infants, with prevalence approaching 70% in the ELBW (≤999g) (1,18-20). In this study, the ELBW infants accounted for only 13.9% of the thrombocytopaenia in our unit (Table 2.1). Developed countries, where the above studies were done, are better equipped to manage ELBW babies and therefore there might be higher birth rates of this category compared to the developing countries, thereby explaining the lower incidence of thrombocytopaenia found in the ELBW infants admitted to our unit during the study period.

Analysis of sepsis (defined as a positive blood culture in a neonate with clinical signs of infection) (17) at the time of diagnosis of thrombocytopaenia in our unit revealed that 20 infants (18.5%) were diagnosed with confirmed blood culture bacterial and fungal sepsis (Table 2.4), including 4 infants (20%) who demised. Of these, confirmed blood culture of gram negative sepsis was predominant, 55% (n=11), with a mortality rate of 36.4% (n=4) (Table 2.9). This finding is consistent with that made by Iqbal et al (2013), who found that thrombocytopaenia is often associated with higher mortality in gram negative sepsis (11). Similarly, Ree et al (2017) in their cohort study of 460 neonates with proven culture positive sepsis, concluded that infants with gram negative sepsis compared to gram positive sepsis have a 6-fold risk of mortality (13).

Table 2.5 shows an overview of severity of thrombocytopaenia in the confirmed septic neonates, with majority (35%), having moderate thrombocytopaenia. Only 15% (n=3) of the septic neonates were severely thrombocytopaenic, whereas 25% (n=5) had platelet counts ≤20 x 10^9/L (very severe thrombocytopaenia). These findings slightly differ from those made by Ree et al (2017) whose study showed that 20% of the neonates with proven culture positive sepsis presented with

very severe to severe thrombocytopaenia (13).

In this study, 9 infants (8.3%) had congenital abnormalities with thrombocytopaenia (Table 2.6). Six of these infants' congenital abnormalities were confirmed, including 3 neonates with confirmed Trisomy 21. Equally, 3 babies were phenotypically described as being dysmorphic without a further diagnosis available (Table 2.6).

Sixteen (14.8%) of the infants diagnosed with thrombocytopaenia developed IVH, of these, more than half had IVH grade II, 56.3% (n=9), while 31.3% (n=5) were not graded (Table 2.7).
Seven of the neonates diagnosed with IVH in the unit (43.8%) had moderate thrombocytopaenia, with majority, 62.5% (n=10) being of late onset. Only 1 (6.3%) and 2 (12.5%) had severe and very severe thrombocytopaenia respectively. Of the 16 thrombocytopaenic neonates complicated with IVH, 43.8% (n=7) mortality was recorded in the unit. Of the IVH group, only 3 (18.8%) received platelet transfusions in the unit, with poor outcome, as all demised (Table 2.7). Overall, of the 108 infants studied, only 3.7% (n=4) received platelet transfusion in our unit.
Comparably, NEC was seen in 15.7% (n=17), with 3 deaths (17.6%) occurring in this group. Five (29.4%) of the NEC infants were confirmed septic at the time of diagnosis with thrombocytopaenia, majority being gram negative or fungal sepsis (Table 2.8).

Of the 108 infants confirmed with thrombocytopaenia, only 3.7% (n=4) received platelet transfusion in our unit, with a mortality rate of 75% (n=3). All 3 infants that demised had IVH grade II before platelet transfusion, which might explain the underlying severity of their respective conditions which might have led to the high mortality observed in this group. In their retrospective study on 134 neonates, Ulusoy et al (2013) also confirmed of high mortality in the thrombocytopaenic neonates treated with platelet transfusion (12). The authors however concluded that it was not clear whether or not this high mortality rate was due to the direct effect of the low platelet count itself or due to the underlying conditions causing severe thrombocytopaenia.

The most common maternal characteristics found in the neonates with thrombocytopaenia in the unit, described in literature as risk factors for the development of neonatal thrombocytopaenia, were PET 29.6% (n=32), and PIH 21.3% (n=23) (Table 2.10). Eslami et al (2013) in their retrospective study of 350 neonates admitted in their NICU, also identified PIH as a significant

maternal risk factor that led to thrombocytopaenia (10). Goyal et (2017) however, had a disparate view, as they did not find any maternal risk factors in their study for neonatal thrombocytopaenia (7). In the study by Saini et al (2017), premature rupture of membranes (PROM) (20.5%), and antepartum haemorrhage (APH) (17.9%) were the leading maternal factors for neonatal thrombocytopaenia, while PIH contributed to only 4.9% (3).

Other outcomes measured revealed that 87% (n=94) of infants were alive at discharge vs 13% (n=14) who had demised. Table 2.9 shows a graphic description of the mortality across each category in the unit. The study showed that infants with birth asphyxia and thrombocytopenia were most likely to demise, 39.3% (n=11), followed by those complicated by IVH, 25% (n=7). According to the severity of thrombocytopaenia among the mortality group, the study revealed that infants with moderate thrombocytopaenia had the highest mortality rate, 42.9% (n=6), followed by infants with mild thrombocytopaenia, 28.6% (n=4). Lower mortality rate was observed in neonates with severe and very severe thrombocytopaenia with each recording equal number of deaths, 14.3% (n=2).

The strength of this study is the study design which was structured to answer the primary objective question. However, because of the retrospective nature of the study design, and the fact that not all infants diagnosed with thrombocytopaenia were included in the study due to missing or incomplete medical records the results of this study should be interpreted with care.
Secondly, even though only infants admitted to the unit within 72 hours of birth were included in the study, some of them only had initial FBCs taken after 3 days of life, and thus it cannot be proven when the thrombocytopaenia occurred, making classification according to timing of thrombocytopaenia (early vs late) difficult, if not impossible. A further study looking at serial FBCs taken timeously is therefore required to prove the timing of thrombocytopaenia. The study described some neonatal and maternal/perinatal conditions found in the neonates diagnosed with thrombocytopaenia, but since this is not a cross-sectional or case-controlled study, the causality could not be measured, and thus a further study is required to elucidate the causal relationship between these factors and the risk of development and severity of thrombocytopaenia in neonates.

2.6. CONCLUSION

Besides anaemia, thrombocytopaenia is the most common haematological abnormality found in the neonates admitted in a neonatal intensive care unit. This is the first study focusing on the prevalence of thrombocytopaenia in neonates admitted to neonatal high care unit in Bloemfontein, Free State province, South Africa. The study confirms that thrombocytopaenia is a common problem in our unit with a prevalence of 15.6%, which is lower than reported in the literature. This difference could be due to differences in the study populations.

This study also shows that the commonest type of thrombocytopaenia in the unit, as reported in the literature, is mild and of early onset. The study describes many neonatal and maternal/perinatal conditions found with thrombocytopaenic infants in the unit, which if minimised, may consequently, eliminate the life-threatening complications of thrombocytopaenia and the need for platelet transfusion. But since this is not a cross-sectional or case-controlled study, the causality could not be measured, and thus further studies are required to elucidate the causal relationship between these factors and the risk of development and severity of thrombocytopaenia in neonates.

Further limitations encountered in the study are the phenotypic description of some babies as being dysmorphic without the actual confirmation of their specific conditions, and the failure to grade some of the babies with IVH, making the actual categorisation of these babies difficult thereby undermining the actual incidence of thrombocytopaenia in the respective group. Also, allo-immune thrombocytopaenia was not specified in the study since none of the patients' files studied documented consideration of this possibility. Therefore, no baby was investigated for allo-immune thrombocytopaenia.

2.7. REFERENCES:

1. Carr R, Kelly AM, Williamson LM. Neonatal thrombocytopenia and platelet transfusion - A UK perspective. Neonatology. 2015;107(1):1–7.

2. Sparger K, Deschmann E, Sola-Visner M. Platelet Transfusions in the Neonatal Intensive Care Unit. Clin Perinatol. 2015;42(3):613–23.

3. Saini R, Saini P, Sehra RN, Saini L. Thrombocytopenia burden and its associating Risk factors: A cross-sectional study at a tertiary care set up. 2017;(7):237–43.

4. Tirupathi K, Swarnkar K, Vagha J. Study of risk factors of neonatal thrombocytopenia. Int J Contemp Pediatr. 2017;4(1):191–6.

5. Ayadi I.D, Ben Hamida E, Youssef A, Sdiri Y, Marrakchi Z. Prevalence and outcomes of thrombocytopenia in a neonatal intensive care unit. La Tunisie Medicale. 2016;94(4):305–8.

6. Kalagiri R, Choudhury S, Carder T, Govande V, Beeram M, Uddin M. Neonatal Thrombocytopenia as a Consequence of Maternal Preeclampsia. Am J Perinatol Reports [Internet]. 2015;6(1): e42–7. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0035-1565923.

7. Goyal P, Gupta S, Natani BS, Agarwal A, Bhatia S. Maternal and Neonatal Risk Factors for Thrombocytopenia in Preterm Infants. Indian Journal of Basic and Applied Medical Research 2017;6(2):39–46.

8. Fustolo-Gunnink SF, Vlug RD, Heckman EJ, Pas AB, Fijnvandraat K, Lopriore E. Early-onset thrombocytopenia in small-for-gestational-age neonates: A retrospective cohort study. PLoS One. 2016;11(5):1–11.

9. Boutaybi N, Razenberg F, Smits-Wintjens VEHJ, van Zwet EW, Rijken M, Steggerda SJ, et al. Neonatal Thrombocytopenia after Perinatal Asphyxia Treated with Hypothermia: A Retrospective Case Control Study. Int J Pediatr [Internet]. 2014; 2014:1–6. Available from: http://www.hindawi.com/journals/ijpedi/2014/760654/.

10. Eslami Z, Lookzadeh MH, Noorishadkam M, Hashemi A, Ghilian R, Pirdehghan A. Thrombocytopenia and Associated Factors in Neonates Admitted to NICU during Years 2010_2011. Iran J Pediatr Hematol Oncol [Internet]. 2013;3(1):205–15. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3915438&tool=pmcentrez&rendertype=abstract.

11. Iqbal Q, Bashir C, Mushtaq S, Ahmad A, Baba AR. Thrombocytopenia and other hematological

parameters in culture positive neonatal sepsis and their impact. J Pediatr Infect Dis. 2013;8(1):25–9.

12. Ulusoy E, Tüfekçi Ö, Duman N, Kumral A, Irken G, Ören H. Thrombocytopenia in neonates: Causes and outcomes. Ann Hematol. 2013;92(7):961–7.

13. Ree IMC, Fustolo-Gunnink SF, Bekker V, Fijnvandraat KJ, Steggerda SJ, Lopriore E. Thrombocytopenia in neonatal sepsis: Incidence, severity and risk factors. PLoS One. 2017;12(10):1–11.

14. Cremer M, Sallmon H, Kling PJ, Bührer C, Dame C. Thrombocytopenia and platelet transfusion in the neonate. Semin Fetal Neonatal Med [Internet]. 2016;21(1):10–8. Available from: http://dx.doi.org/10.1016/j.siny.2015.11.001.

15. Sparger KA, Assmann SF, Granger S, Winston A, Christensen RD, Widness JA, et al. Platelet transfusion practices among very-low-birth-weight infants. JAMA Pediatr. 2016;170(7):687–94.5.

16. Sola-Visner M, Bercovitz RS. Neonatal Platelet Transfusions and Future Areas of Research. Transfus Med Rev [Internet]. 2016;30(4):183–8. Available from: http://dx.doi.org/10.1016/j.tmrv.2016.05.009

17. Boutaybi N, Steggerda SJ, Smits-Wintjens VEHJ, van Zwet EW, Walther FJ, Lopriore E. Early-onset thrombocytopenia in near-term and term infants with perinatal asphyxia. Vox Sang. 2014;106(4):361–7.

18. Bolat F, Kiliç SÇ, Oflaz MB, Gülhan E, Kaya A, Güven AS, et al. The prevalence and outcomes of thrombocytopenia in a neonatal intensive care unit: A three-year report. Pediatr Hematol Oncol. 2012;29(8):710–20.

19. Tiller H, Husebekk A, Ahlen MT, Stuge TB, Skogen B. Current perspectives on fetal and neonatal alloimmune thrombocytopenia - Increasing clinical concerns and new treatment opportunities. Int J Womens Health. 2017; 9:223–34.

20. Yurdakök M. Immune thrombocytopenia in the newborn. 2017;6(1):1–10.

21. Chakravorty S, Roberts I. How I manage neonatal thrombocytopenia. Br J Haematol. 2012;156(2):155–62.

22. Holzhauer S, Zieger B. Diagnosis and management of neonatal thrombocytopenia. Semin Fetal Neonatal Med [Internet]. 2011;16(6):305–10. Available from: http://dx.doi.org/10.1016/j.siny.2011.07.008.

23. Joolay, Yaseen; Horn, Alan; Raban, M. Shukri; Harrison, Michael; Tooke, Lloyd; Rhoda N. Neonatal guidelines and drug doses. 1st ed. Joolay, Yaseen; Horn A, editor. Cape Town: The Science Press; 2012. 78 p.

24. Wittenberg D. Coovadia's paediatrics & child health. 6th ed. Coovadia H, editor. Cape Town: Oxford University Press Southern Africa (Pty) Ltd; 2009. 120 p.

25. Cloherty, John P; Eichenwald, Eric C; Hansen, Anne R; Stark AR. Manual of Neonatal Care. 7th ed. Cloherty, John P; Eichenwald, Eric C; Hansen, Anne R; Stark AR, editor. Philadelphia: Lippincott Williams & Wilkins; 2012. 154 p.

26. Gomella, Tricia Lacy; Cunningham, M. Douglas; Eyal FG. NEONATALOGY Management, Procedures, On-Call Problems, Diseases, and Drugs. 7th ed. Gomella, Tricia Lacy; Cunningham, M. Douglas; Eyal FG, editor. New York: McGraw Hill Education LLC; 2013. 154p.

3. APPENDICES:

3.1 APPENDIX A: Letter of approval from HSREC:

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

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

Dear **Dr Anselm Onwugbolu**

09-Nov-2018

Ethics Clearance: **Prevalence of thrombocytopaenia in neonates admitted in neonatal high care unit of Pelonomi Academic Hospital Bloemfontein between January 2017 and June 2017**

Principal Investigator: **Dr Anselm Onwugbolu**

Department: **Paediatrics and Child Health Department (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.
Your ethical clearance number, to be used in all correspondence is: **UFS-HSD2018/1026/2711**
The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.
We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.
A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.
The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.
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 and we wish you every success with your research. 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

3.2 APPENDIX B: Permissions from DOH and NHLS:

health
Department of Health
FREE STATE PROVINCE

22 October 2018

Dr A Onwugbolu
Dept. of Paediatrics and Child Health
UFS

Dear Dr A Onwugbolu

Subject: Prevalence of thrombocytopaenia in neonates admitted in neonatal high care unit of Pelonomi Academic Hospital Bloemfontein between January 2017 and June 2017

- 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 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 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: 31/10/18

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

NATIONAL HEALTH LABORATORY SERVICE

Office of the Free State Business Manager
Pelonomi Academic Hospital
120 Dr Belcher Road
Heidedal, Bloemfontein
9301

Tel no.: 051- 405 9348
Fax no.: 051- 405 9357

22 August 2018

REQUEST FOR APPROVAL OF LABORATORY DATA FOR ACADEMIC PURPOSE

Requestor: Dr Anselm Uche Onwugbolu

Project Name: Prevalence of thrombocytopaenia in neonates admitted in neonatal high care unit of Pelonomi Tertiary Hospital Bloemfontein between January 2017 and June 2017

Dear AU Onwugbolu

Your request for use of laboratory data and information for your research study mentioned above is hereby granted under the following conditions:

1. That University Ethical Committee approval is obtained
2. All exiting laboratory data remain confidential to the patient and doctor
3. **Free State Department of Health gives approval**
4. NHLS is notified before any publications of any results/findings is made
5. NHLS is recognized in all publications

Hope you find this in order

Regards

MS. NOMA MADUNA
MANAGER (BUSINESS) - FREE STATE

3.3 APPENDIX C: Permission from HOD:

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

20 July 2018

Prof A Venter
Prof/Head Clinical Department, Paediatrics and Child Health

Dear Prof Venter,

RE: APPLICATION TO CONDUCT AN MMED RESEARCH PROJECT AT PELONOMI ACADEMIC HOSPITAL

Protocol Title: Prevalence of thrombocytopaenia in neonates admitted in neonatal high care unit of Pelonomi Academic Hospital Bloemfontein between January 2017 and June 2017

Study Supervisor: Dr Jacobus van Rooyen

Hereby I, Dr A Onwugbolu, would like to request your approval to perform the above-mentioned research project in fulfilment of the MMed degree at Pelonomi Academic Hospital. Please find attached a copy of the protocol for this study.

Yours Sincerely

Dr AU Onwugbolu

Permission granted.
20/7/2018.
Prof A Venter
MB ChB, DCH, FCP, M.Med (Paed), PhD
Dept Paediatrics

Department of Paediatrics and Child Health / Departement Pediatrie en Kindergesondheid
205 Nelson Mandela Drive/Rylaan | Park West/Parkwes | Bloemfontein 9301 | South Africa/Suid-Afrika
P.O. Box/Posbus 339 (G69) | Bloemfontein 9300 | South Africa/Suid-Afrika | www.ufs.ac.za | F: +27(0)51 444 3230
Prof A Venter: T: +27 (0)51 405 3181 | E: Gnpdav@ufs.ac.za / Prof DK Stones: T: +27 (0)51 405 2820 | E: StonesDK@ufs.ac.za
Prof SC Brown: T: +27 (0)51 405 3254 | E: Gnpdscb@ufs.ac.za / Dr A van der Vyver: T: +27 (0)51 405 3184 | E: Gnpdavdv@ufs.ac.za

3.4 APPENDIX D: Research Protocol:

RESEARCH PROTOCOL

Prevalence of thrombocytopaenia in neonates admitted in neonatal high care unit of Pelonomi Tertiary Hospital Bloemfontein between January 2017 and June 2017

By

Dr Anselm Uche Onwugbolu, MBBS

Protocol for a mini-dissertation submitted in fulfilment of the requirements for the degree Master of Medicine (MMed) in Paediatrics

in the

Department of Paediatrics and Child Health

Faculty of Health Sciences at the University of the Free State

CANDIDATE

Dr Anselm Uche Onwugbolu MBBS

Registrar: Department of Paediatrics and Child Health

Faculty of Health Sciences

University of the Free State

Student number:

2016433324

STUDY LEADER

Dr J van Rooyen MBchB, MMed (Paeds), FC Paed(SA)

Consultant: Department of Paediatrics and Child Health

Faculty of Health Sciences

University of the Free State

TABLE OF CONTENTS:

LIST OF ACRONYMS:

AGA	-	Appropriate-for-Gestational Age
APH	-	Ante Partum Haemorrhage
DIC	**-**	Disseminated Intravascular Coagulation
ELBW	-	Extremely Low Birth Weight
EOTP	-	Early Onset Thrombocytopaenia
FBC	**-**	Full Blood Count
FNAIT	-	Foetal and Neonatal Alloimmune Thrombocytopaenia
GDM	-	Gestational Diabetes Mellitus

HELLP	-	Haemolysis, Elevated Liver enzymes and Low Platelet count
HIV	-	Human Immunodeficiency Virus
HPA	-	Human Platelet Antigen
ICH	-	Intracranial Haemorrhage
ITP	-	Immune Thrombocytopaenia
IUGR	-	Intrauterine Growth Restriction
IVH	-	Intraventricular Haemorrhage
LOTP	-	Late Onset Thrombocytopaenia
NEC	-	Necrotizing Enterocolitis
NHCU	-	Neonatal High Care Unit
NHLS	-	National Health Laboratory Service
NICU	-	Neonatal Intensive Care Unit
PET	-	Pre-Eclamptic Toxaemia. Also known as Pre-Eclampsia
PIH	-	Pregnancy Induced Hypertension
PROM	-	Premature Rupture of the Membrane
PTH	-	Pelonomi Tertiary Hospital
SGA	-	Small-for-Gestational Age
TAR	**-**	Thrombocytopaenia Absent Radii
TORCH	**-**	Toxoplasmosis, Other (syphilis, varicella zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections
UFS	-	University of the Free State
VLBW	-	Very Low Birth Weight

DEFINITIONS OF SELECTED TERMS:

Appropriate for gestational age:	A birth is considered to be appropriate for gestational age if the birth weight is between the 10th and 90th percentiles for the infant's gestational age on the Lubchenco infant's chart (24).
Biostatistics:	Data analysed are derived from the biological sciences and medicine (van Belle, G., Fisher, L.D. Heagerty, P.J. 2004:2).
Birth Asphyxia:	Birth asphyxia occurs when at least three of the following criteria apply: (a) signs of foetal distress before delivery (abnormal cardiotocography recording), (b) Apgar score ≤5 at 5min, (c) arterial pH <7.1 and base excess ≤-16mmol/L or lactate >10mmol/L in either arterial umbilical cord blood sample or capillary blood gas within one hour after birth, (d) respiratory failure requiring resuscitation during at least 5min after birth, (e) multiple organ failure (17).
Extremely low birth weight infants:	Infants born with birth weight less than 1000 grams (14,25).
Intrauterine growth restriction:	A reduction in the expected foetal growth of an infant. A foetus <10^{th} weight percentile for age or a ponderal index <10%. SGA and IUGR are related but not synonymous (26).
Neonate:	Baby at the first twenty-eight days of life (24).
Pre-Eclampsia:	Hypertension with proteinuria in pregnant women after 20 weeks' gestation (26).

Premature infants:	Infants born before the start of 37 weeks' gestation (259 days) (24).
Small for gestational age:	Birthweight 2 standard deviations below the mean weight for gestational age or below the 10th percentile for gestational age (13).
Very low birth weight infants:	Infants born with weight less than 1500 grams (13,14).

LIST OF TABLES:

LIST OF FIGURES:

SUMMARY:

Thrombocytopaenia is the most common haematological abnormality, besides anaemia, found in the newborn infants admitted in a neonatal intensive care unit (2). Even though most cases are mild or moderate, requiring minimal or no intervention, and resolving spontaneously within the first week of life; a few infants, especially the very sick ones or the extremely low birth weights (<1000g), may present with severe and in some cases persistent thrombocytopaenia, which will require clinical intervention including platelet transfusion (1-12).

Causes of thrombocytopaenia in the neonates are multifactorial. Maternal conditions, especially those resulting in placental insufficiency, as well as foetal factors have all been implicated.

Early onset thrombocytopaenia (occurring ≤72hrs of life) usually results from maternal conditions such as gestational diabetes or drug use, or from complications of pregnancy such as intrauterine growth restriction, HELLP syndrome as well as chronic foetal hypoxia. Conversely, late onset thrombocytopaenia (occurring >72hrs of life) is almost always a result of sepsis or necrotizing enterocolitis (1–12). Thrombocytopaenia occurring in these conditions are usually severe in nature warranting multiple platelet transfusions (1,10).

Complications posed by severe thrombocytopaenia in preterm infants admitted in a neonatal intensive care unit make up part of the global burden of neonatal morbidity and mortality faced by neonatologists.

Currently, there is significant disagreement among practitioners with regards to the platelet count below which a new born infant with thrombocytopaenia should be transfused (2), especially in light of lack of evidence-based recommendations among neonatologists worldwide which favour or disfavour platelet transfusions in neonates. Decisions whether or not to transfuse are basically expert opinions in any given institution (1,2,14). Despite this dilemma however, appropriate diagnostic and therapeutic management guidelines are necessary to prevent death or neurological sequelae in the severely thrombocytopaenic infants.

This study aims to investigate and determine the prevalence of thrombocytopaenia in the neonates admitted to the neonatal high care unit of Pelonomi Academic Tertiary in Bloemfontein, Free State province of South Africa, and also to determine the maternal and neonatal risk factors contributing to thrombocytopaenia in the unit.

1. Introduction

Evaluation of a neonate with thrombocytopaenia is a challenge to neonatologists in both developed and developing countries. This is evidenced by lack of common agreement regarding management guidelines of neonatal thrombocytopaenia (1,2). Developing a workable diagnostic strategy regarding the evaluation of these neonates with thrombocytopaenia is key towards prevention of death or neurological sequelae in the severely thrombocytopaenic infants.

Whereas mild to moderate thrombocytopaenia (platelet count 51-149 x 10^9/L), can resolve spontaneously within the first ten days of life, requiring no clinical intervention, infants with severe thrombocytopaenia (platelet count 21-50 x 10^9/L) will require proper clinical work-ups and in some cases, multiple platelet transfusions (1,10).

2. Background

2.1. Literature Review

2.1.1. Definition of neonatal thrombocytopaenia

Several studies in the past had engaging conclusive findings with regards to the genesis of platelet production in the foetuses. Some existing data have shown that production of platelets in foetuses can commence as early as five weeks after conception and that by the end of the second trimester life, foetuses actually have estimated normal postnatal platelet range of 150 - 450 x10^9/L, which is the same range observed in older children and adults (1,13).

Thus, the definition of thrombocytopaenia is the same for preterm and term neonates, as it is in adults, which is a platelet count of < 150 x 10^9/L (1-17). Neonatal thrombocytopaenia can be classified based on the timing of presentation after birth into "early onset" (occurring ≤ 72hrs of life) and "late onset" (occurring >72hrs of life) (1-12). Based on severity, the thrombocytopaenia can be mild (platelet count of 101-149 x 10^9/L), moderate (platelet count of 51-100 x 10^9/L), severe (platelet count of 21-50 x 10^9/L), or very severe (platelet count of ≤20 x 10^9/L) (8,13).

2.1.2. Prevalence of neonatal thrombocytopaenia

Thrombocytopaenia is one of the most common haematological problems in the newborns (2-5,10,12-14,17). Researchers however differ as far as its prevalence in both healthy and sick neonates. In healthy neonatal population, Eslami et al (2013) reported an occurrence of 1-2% (10), whereas lower prevalence has been reported by other authors (7,12,16). A handful of authors have reported a higher occurrence of 1-5% in healthy neonates (4,8,9,17). The prevalence however was reportedly much higher in sick neonates admitted to a neonatal intensive care unit (NICU), again, with considerable disagreement between the researchers. While some reviews reported a prevalence of 18-35% (2,10,14,15) in sick neonates, others reported a prevalence of 22-35% (1,4,8,9,13,17). In their cohort study of thrombocytopaenia in neonatal sepsis, Ree et al (2017) found an incidence of 49% of

thrombocytopaenia in all neonates with proven culture positive sepsis admitted to a tertiary NICU (13). In the same study, the authors found that 20% of the neonates with confirmed sepsis had severe (platelet count 21-50 x 10^9/L) and very severe thrombocytopaenia (platelet count ≤20 x 10^9/L) (13). The reason for this significant variations in the prevalence and incidence of neonatal thrombocytopaenia reported among researchers may be due to the different populations of these studies (19).

The common thread in all of the studies however, is the finding that the prevalence is higher in sick neonates than in healthy population.

Researchers also found that the prevalence varied depending on the gestational age (GA) and birth weights of the infants, with the prevalence approaching 70% in the extremely low birth weight infants (<1000g) (1,18–20). Studies have shown that besides GA and sepsis, high incidence of thrombocytopaenia can be found in neonates with other conditions. For instance, in their retrospective case control studies, Boutaybi et al (2014) found that the vast majority (80%) of neonates with perinatal asphyxia treated with therapeutic hypothermia developed thrombocytopaenia (9). The prevalence however, was reportedly low in some studies. In the study of Bolat et al (2012), the prevalence was 9.4% (18).

In the same study Bolat et al (2012) found that 97 of the 208 neonates studied (46.6%) had mild thrombocytopaenia, 64 neonates (64/208 = 30.8%) had moderate thrombocytopaenia, 33 neonates (33/208 = 15.9%) had severe, and 14 neonates (14/208 = 6.7%) had very severe thrombocytopaenia (18). In contrast, Ayadi et al (2016) concluded that of the 808 neonates admitted to their NICU, mild thrombocytopaenia was found in 22.3%, moderate in 36.7%, and severe in 41% (5).

2.1.3. Causes of neonatal thrombocytopaenia

2.1.3.1. Congenital causes

When the mother produces IgG antibodies against foetal antigens (alloantigens) inherited from the father, these allo-antibodies can cross the placenta and destroy the foetal platelets resulting in foetal and neonatal alloimmune thrombocytopaenia (FNAIT) (1,2,19,20). Also, a mother with immune thrombocytopaenic purpura (ITP) can produce autoantibodies which transplacentally destroy the foetal platelets resulting in immune thrombocytopaenia (1,2,20). FNAIT occurs in approximately 1 in 1000 live births (19,20) and is the leading cause of early onset moderate or severe thrombocytopaenia in otherwise healthy appearing neonates (2,14,19,20).

Congenitally acquired infections such as TORCH (toxoplasma gondii, rubella, cytomegalovirus and herpes simplex virus), enterovirus and human immunodeficiency virus (HIV), can all cause foetal and neonatal thrombocytopaenia (6,14).

Genetic disorders, e.g. trisomy 13, 18, and 21 can also cause thrombocytopaenia in the neonates (6).

2.1.3.2. Acquired causes

The most frequent acquired causes of early onset neonatal thrombocytopaenia are chronic foetal hypoxia, resulting from intrauterine growth restriction (IUGR), and maternal disorders such as maternal diabetes, pre-eclampsia/pregnancy-induced hypertension (PIH), or HELLP syndrome (1,2,6,8,10-12,14,17,18). The resulting thrombocytopaenia is usually mild and self-limiting (14) (Table 1). On the other hand, late onset thrombocytopaenia, usually severe, is almost always caused by sepsis or NEC (1,2,6,8,10-12,14,17,18) (Fig.1).

Table 1.1: Aetiologies of early-onset neonatal thrombocytopaenia (14).

Severity		Mild-to-moderate thrombocytopaenia, rarely severe
Course		Slow, self-limiting
Causes		
	Frequent	**Maternal/placental** • Pre-eclampsia/eclampsia, placental insufficiency, HELLP syndrome, hypertension, gestational diabetes • Rhesus haemolytic disease In case of severe thrombocytopaenia • Bacterial infection • Asphyxia • Congenital, mostly viral infections • Alloimmune thrombocytopaenia • Alloimmune hepatitis
	Rare	**Congenital** • Trisomy 13, 18, 21 • Thrombocytopaenia absent radii syndrome • Congenital amegakaryocytic thrombocytopaenia • Wiskott-Soulier syndrome • Bernard-Soulier syndrome • MYH9-associated diseases • Inborn errors of metabolism (Gaucher disease, methylmalonic acidaemia) • Kasabach Merrit, haemangioendothelioma

HELLP: haemolysis, elevated liver enzymes, low platelet count.

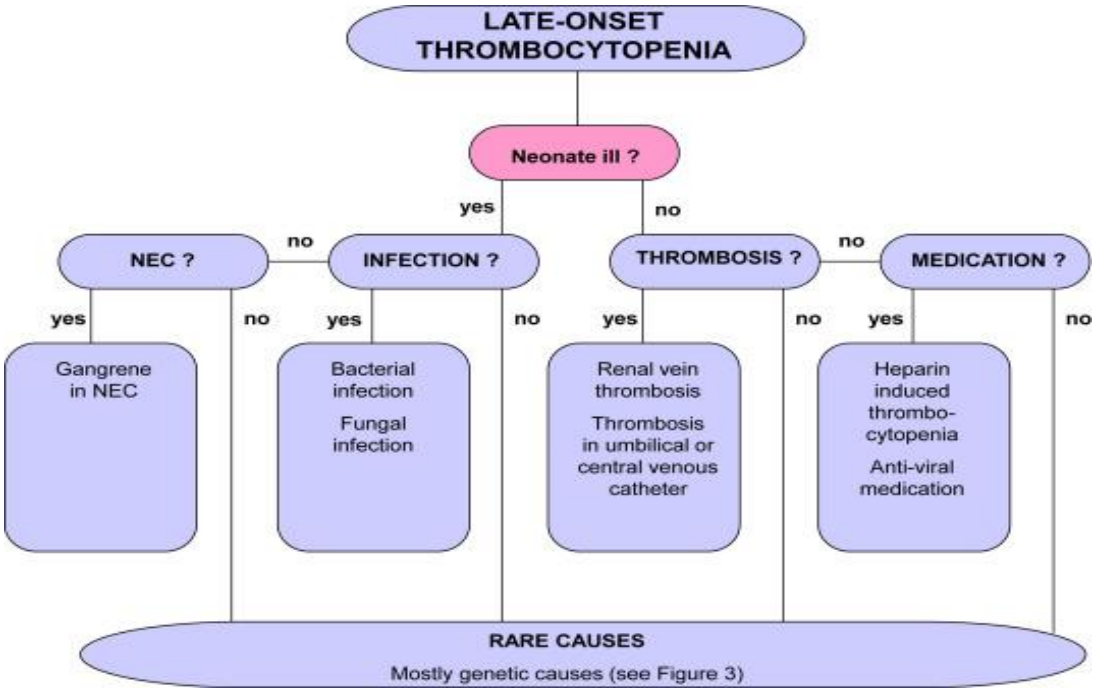


Fig. 1.1 (14). Algorithm for neonatal late-onset thrombocytopaenia (>72h postnatal age) when considering the differential diagnosis. NEC, necrotizing enterocolitis.

2.1.4. Mechanisms of neonatal thrombocytopaenia

Three different kinetic mechanisms responsible for thrombocytopaenia in neonates as well as in adults have been described. These include (12,18):

(iv) Decreased platelet production (e.g. thrombocytopaenia seen in babies born to mothers with severe placental insufficiency);
(v) Increased platelet destruction (seen in thrombocytopaenia of sepsis or NEC);
(vi) Platelet sequestration (mostly secondary to hypersplenism);

Or a combination of these processes, which in some reports is identified as the number four mechanism.

3. Thrombocytopaenia and associated risk factors in neonates

The risk factors associated with the development of thrombocytopaenia in neonates can be divided into maternal and neonatal factors. Many authors have linked multiple neonatal and maternal conditions as causative factors of thrombocytopaenia in the neonates admitted to NICU.

3.1. Maternal risk factors

In their retrospective one-year period of study of 350 neonates admitted to their NICU, of which 100 (28.5%) had thrombocytopaenia, Eslami et al (2013) analysed the contributory

effects of both maternal and neonatal variables and concluded that the significant maternal risk factors that led to thrombocytopaenia in the neonates studied were pregnancy induced hypertension (PIH), gestational diabetes mellitus (GDM), eclampsia and autoimmune disease such as idiopathic thrombocytopaenia (ITP) (10). The results showed that the most common maternal risk factor was PIH (Table 1.2).

Table 1.2: Incidence maternal risk factors in thrombocytopaenic neonates (10).

Maternal Risk Factor	Frequency	
	N	%
PIH	13	46.4
GDM	9	32.1
GDM+PIH	3	10.7
Eclampsia	2	7.1
ITP	1	3.6

GDM: gestational diabetes mellitus; ITP: immune thrombocytopaenic purpura; PIH: pregnancy induced hypertension.

Similar findings were made by other researchers in their various studies (4,5). In contrast to most studies, Goyal et al (2017), did not find any maternal risk factors in their study for neonatal thrombocytopaenia (7). In another study by Saini et al (2017), premature rupture of membranes (PROM) (20.5%), and antepartum haemorrhage (APH) (17.87%) were the leading maternal causative factors for neonatal thrombocytopaenia, while PIH contributed to only 4.94% (3).

3.2. Neonatal risk factors

Several studies have shown significant association between multiple neonatal variables like sepsis, birth asphyxia, and low birth weight (LBW), intrauterine growth restriction (IUGR), small-for-gestational age (SGA), ABO incompatibility, and necrotising enterocolitis (NEC) and neonatal thrombocytopaenia. Eslami et al (2013) found out that the most common neonatal risk factor for thrombocytopaenia was sepsis (31.9%), followed by IUGR (20.8%) (Table 1.3) (10).

Table 1.3. Incidence neonatal risk factor in thrombocytopaenic neonates (10).

Neonatal Risk Factor	Frequency	
	N	%
Sepsis	23	31.9
IUGR	15	20.8
Asphyxia	10	13.9
Asphyxia + IUGR	5	6.9
Sepsis + IUGR	2	2.8
Asphyxia + Sepsis	1	1.4
NEC	2	2.8
ABO	3	4.2
Other	11	15.3
Sum	72	100

ABO: blood group system type A, B, O; IUGR: intrauterine growth restriction; NEC: necrotizing enterocolitis.

These findings were similar to other studies (4,5,7,13). Some researchers however differ in their observations concerning causal relationship between these neonatal conditions and thrombocytopaenia. Fustolo-Gunnik et al (2016) linked early-onset thrombocytopaenia to SGA neonates (8). In the same comparative study, the authors found that SGA neonates were at increased risk of early-onset thrombocytopaenia compared to their appropriate gestational age (AGA) counter parts. Boutaybi et al (2014) did a retrospective case control study on neonates with perinatal asphyxia treated with therapeutic hypothermia, and found that 80% of the study population had thrombocytopaenia (9). In a different study by Boutaybi et al (2014), perinatal asphyxia was found to be a risk factor in 51% of cases for early-onset neonatal thrombocytopaenia (17). LBW, poor APGAR scores, prematurity and birth asphyxia were the causative factors found by Saini et al (2017) in their cross-sectional study at a tertiary care set up (3).

4. Diagnostic approach to neonatal thrombocytopaenia

4.1. Clinical history

In every neonate with suspected thrombocytopaenia, literatures have shown that most of the possible causes can be determined by the clinical history and presentation. Thorough neonatal history (gestational age, birth weight, timing of onset) as well as maternal history including present and past pregnancies, and family history of hereditary thrombocytopaenia, are therefore advisable (21,22).

4.2. Laboratory investigations

Full blood count (FBC), including peripheral blood smears, can be done to ascertain the platelet count and to distinguish between macro-and micro-thrombocytopaenia respectively

(22). The infants should also be screened for any suspected specific disease including sepsis. The data collected from the clinical history and laboratory investigations can then be incorporated into a diagnostic algorithm for preterm (Fig. 2) and term (Fig. 3) neonates below (20).

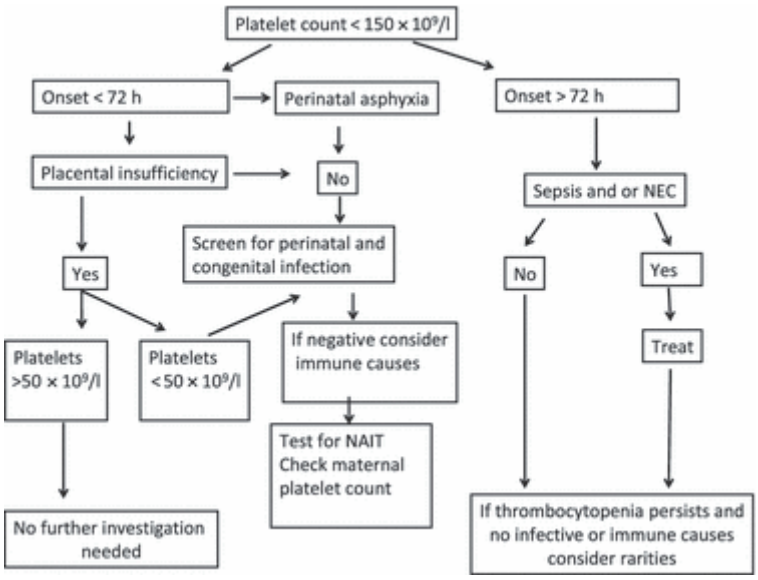


Fig.1.2 (20). Diagnostic algorithm for investigation of preterm neonates with thrombocytopaenia. NEC, necrotizing enterocolitis; NAIT, neonatal alloimmune thrombocytopaenia.

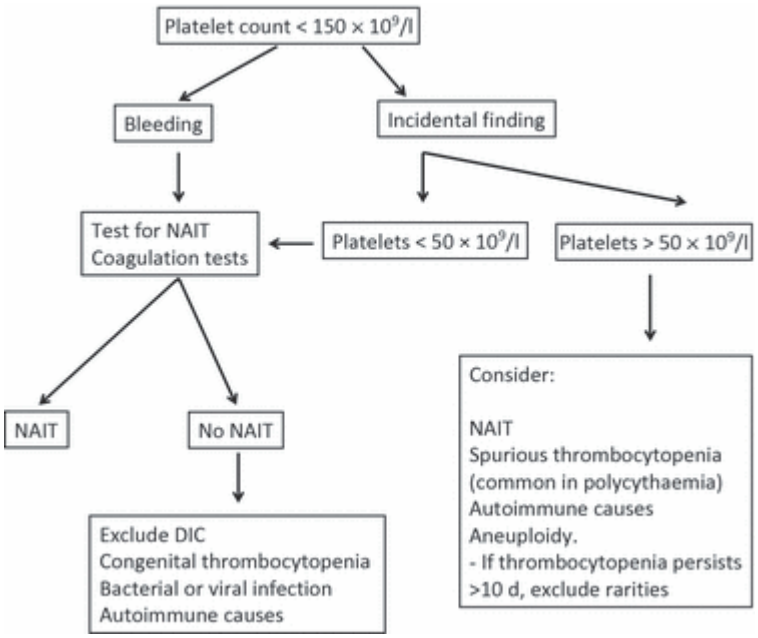


Fig.1.3 (20). Diagnostic algorithm for investigation of term neonates with thrombocytopaenia. NAIT, neonatal alloimmune thrombocytopaenia; DIC, disseminated intravascular coagulation.

5. Management of thrombocytopaenia in the neonates

Besides supportive measures, the only management of thrombocytopaenia is platelet transfusion (6). There is a widespread agreement among researchers regarding the paucity of evidence-based practice in neonatal thrombocytopaenia and platelet transfusion thresholds in infants admitted to NICU (1,2,6,14-16). This has created a wide variation in practice among neonatologists. For instance, prophylactic platelet transfusions are being given liberally at higher platelet thresholds in the USA and Canada compared to Europe (1,14-16).

5.1. Threshold for platelet transfusion

There is a great deal of variability regarding the platelet count below which a new born infant with thrombocytopaenia should be transfused, especially in light of lack of evidence-based recommendations among neonatologists worldwide (1,2,14-16,21,22). Hence, decisions whether or not to transfuse are based on expert opinions in any given institution (1,2). Be that as it may, a reasonably safe threshold for platelet transfusion for most neonates have been reported by various authors to be 20 x 10^9/L following findings which confirmed that most neonates whose platelet counts fell below 20 x 10^9/L did not develop major haemorrhage like intraventricular haemorrhage (IVH), pulmonary haemorrhage, gastrointestinal (GI) haemorrhage (6,20).

5.2. Platelet transfusion guidelines

Confusion exists among neonatologists all over the world concerning platelet count thresholds used as triggers for platelet transfusion in neonates with severe thrombocytopaenia. The fact that no data exist yet which show whether or not liberal vs more restrictive platelet transfusion strategy has any influence on the outcome of severe thrombocytopaenia (1), is more confounding than helpful. Internationally, neonatologists are awaiting the outcome of the large multicentre randomised trial currently being conducted in several European countries by The Platelets for Neonatal Transfusion- study 2 (PlaNeT-2). This study compares liberal vs restrictive prophylactic platelet transfusion strategies (50 vs 20 x 10^9/L, respectively) in preterm infants with thrombocytopaenia (14,16). The initiative, will no doubt be of great benefits if and when evidence-based standardised guidelines are formulated following this study. Until then, decision whether or not to transfuse is based on expert opinion (1). Reports have shown that whereas practices

in UK and other European countries favour restrictive platelet transfusion strategy, US and Canadian neonatologists generally favour more liberal transfusion thresholds (1,14-16).

5.2.1. Guidelines in our local practice

There are no published existing platelet transfusion guidelines in the Free State province. The Standard Treatment Guidelines and Essential Medicines List for South Africa, Hospital Levels Paediatrics (EDL) (2017) do not discuss much on neonatal thrombocytopaenia and definitely no discuss was made on the transfusion guidelines. In our local practice at Pelonomi Academic Hospital, the decision whether or not to transfuse neonates with severe thrombocytopaenia with platelets is at the discretion of the consultant on duty. This decision is usually based on the only available guidelines in our area (Table 4) (23), which are recommendations based on the Cape Town practices. According to these guidelines, a platelet count $<30 \times 10^9$/L should form the transfusion threshold even if the neonate is asymptomatic (not bleeding) or not. This means that we may consider *prophylactic* platelets transfusion to all neonates, term or preterm, with a confirmed platelet count of $<30 \times 10^9$/L, and *therapeutically* to neonates who are bleeding or with suspected or confirmed neonatal allo-immune thrombocytopaenia (NAITP) [Table 4].

Table 1.4. Guidelines for platelet transfusion thresholds for neonates (23).

Platelet count (x 10^9/L)	Non-bleeding neonate	Bleeding neonate	*NAITP (proven or suspected)
<30	Consider transfusion in all patients	Transfuse	Transfuse (with **HPA compatible platelets)
30-49	Do not transfuse if clinically stable. Consider transfusion if: • < 1000g and < 1 week of age • Hypotension requiring inotropic support • Previous major bleeding tendency (e.g. Grade 3-4 IVH) • Current minor bleeding (e.g. petechiae, puncture site oozing) • Current coagulopathy • Respiratory disease requiring FiO2 > 40% or MAP > 9cm • Seizures within the last 72 hrs • Requires exchange transfusion • Pre-surgery (within 24 hrs) • Post-surgery (within 5 days)	Transfuse	Transfuse (with HPA compatible platelets)
50-99	Do not transfuse	Transfuse	Transfuse (with HPA compatible platelets if major bleeding present)
>99	Do not transfuse	Do not transfuse	Do not transfuse

* NAITP: neonatal alloimmune thrombocytopaenia

** HPA: human platelet antigen.

With platelet counts 30-49 x 10^9/L, our guidelines advise not to transfuse if the neonate is clinically stable, but to transfuse if the following conditions apply: bleeding, has suspected or confirmed NAITP, < 1000g and < 1 week of age, hypotension requiring inotropic support, previous major bleeding tendency (e.g. Grade 3-4 IVH), current minor bleeding (e.g. petechiae, puncture site oozing), current coagulopathy, respiratory disease requiring FiO2 > 40% or MAP > 9cm, seizures within the last 72 hrs, requires exchange transfusion, pre-surgery (within 24 hrs), or post-surgery (within 5 days). If the infant's platelet count is 50-99 x 10^9/L, transfusion is indicated only if the neonate is symptomatic with bleeding or has suspected or proven NAITP. However, with a platelet count >90 x 10^9/L, we do not transfuse despite the clinical condition of the neonate (Table 4) (23).

6. Possible outcomes of neonatal thrombocytopaenia

6.1. Neonatal thrombocytopaenia and risk of bleeding

Whereas thrombocytopaenia can be a risk factor for haemorrhage, the majority of neonates with thrombocytopaenia do not develop major haemorrhage (11). Studies have shown that

the risk of bleeding in neonates with thrombocytopaenia is highest in the very preterm infants with FNAIT, sepsis and NEC in the first week of life (2,12,22). Bleeding symptoms vary according to the individual neonate, and most of them are mild (22). There can be cutaneous bleed, intracranial, pulmonary or gastrointestinal bleedings. In their retrospective analysis of 134 neonates with thrombocytopaenia, Ulusoy et al (2013) found that 11% had pathological haemorrhage (intracranial haemorrhage 50%, pulmonary haemorrhage 32%, and gastrointestinal haemorrhage 18% (12). These haemorrhages were significantly more common in thrombocytopaenic infants who were premature and with sepsis (12).

6.2. Neonatal thrombocytopaenia and mortality rates

Studies have shown high mortality rates in neonates with severe thrombocytopaenia who received platelet transfusions (12,18). However, it is unclear whether the high mortality is a result of underlying conditions causing severe thrombocytopaenia in the first place or a direct effect of platelet transfusion itself (12). Be that as it may, when compared with neonates without thrombocytopaenia, mortality rates have been found to be high in infants with severe or very severe thrombocytopaenia (18). The mortality is even higher in thrombocytopaenic neonates with confirmed bacterial grand negative or fungal sepsis (11).

6.3. Relationship between severity of thrombocytopaenia and bleeding risk and mortality

There is poor or no significant correlation between the severity of thrombocytopaenia and risk of bleeding and or mortality in neonates with thrombocytopaenia (2,12,15).

7. Problem statement

Beside phlebotomy-induced anaemia, thrombocytopaenia is the commonest haematological abnormality encountered in the infants admitted to NICU. This condition is more prevalent in the very low birth weight and sick premature infants who constitute the highest population in our neonatal high care unit. Hence, neonatal thrombocytopaenia is a problem in our local practice. It is therefore pertinent to have a working diagnostic approach and proper management guidelines when dealing with these sick infants.

Literature studies have highlighted the problem of lack of evidence-based data locally and internationally regarding management guidelines of neonatal thrombocytopaenia and the challenges faced by the clinicians all over the world.

8. Aim of this study

The aim of this study is to determine the prevalence of neonatal thrombocytopaenia in neonates admitted to the neonatal high care unit of Pelonomi Tertiary Hospital (PTH) from January 1st 2017 to June 30th 2017.

9. Objectives of this study

The primary objective of this study is to determine the prevalence of neonatal thrombocytopaenia in neonates admitted to the neonatal high care unit of PTH.

The secondary objectives of this study will be:

(i) To determine the maternal and neonatal risk factors contributing to thrombocytopaenia in the unit.

(ii) To look into the outcomes of the infants diagnosed with thrombocytopaenia in the unit with or without platelet transfusion.

(iii) To determine whether or not there is any significant correlation between severity of thrombocytopaenia and morbidity and mortality in the studied population.

10. Methodology

10.1. Study design

This study will be a descriptive cross-sectional study.

10.2. Study population

The population of this study will consist of all neonates admitted to the NHCU of PTH during January 1st 2017 to June 30th 2017.

Since all infants born and admitted during the study period will be reviewed, the sampling method will not be required.

10.2.1. Sample size

The estimated sample size for this study will be approximately 600 neonates.

10.2.2. Inclusion criteria

- All neonates admitted to the NHCU of PTH within the study period will be included in this study.
- The infants must be born within 72hrs of admission to the neonatal high care unit, because it will be difficult to classify the thrombocytopaenia of the infants admitted at age of 4 or more days.
- Infants born with congenital abnormalities and syndromes will be included.

10.2.3. Exclusion criteria

- Infants admitted to the NHCU of PTH after 72hrs of birth from within or without PTH.
- Infants whose medical files are either missing or incomplete will be excluded.
- Infants without any form of haematological investigation done while being admitted in the NHCU of PTH will also be excluded from the study, e.g. infants with hypoglycaemia admitted for only blood glucose correction and monitoring.

11. Measurement

The candidate will identify all admissions by means of the admission register and go through all the laboratory results via the National Health Laboratory Service (NHLS) trackcare web results viewer. He will then identify those with defined thrombocytopaenia- these will be the sample size, and these patients' medical files will be withdrawn from the registry. Information needed for completion of data form (see Appendix A) will be collected from these files.

Data regarding maternal risk factors for neonatal thrombocytopaenia as elaborated in the literature will be collected from the neonatal admission books. The maternal antenatal history is usually collected and documented as per routine by the doctor admitting any neonate to the NHCU.

The researcher will be the one to collect all this information for proper documentation in the data capture form.

A data capture sheet form pre-designed by the researcher based on the information gathered during the literature review will be used to collect all the necessary information required for this research (see Appendix A).

This information captured in the data form will then be entered in an Excel sheet, and discussed with the department of Biostatistics during the planning phase of the study to ensure that all the necessary and relevant information are collected and that the information will be analysable.

12. Ethics

The research protocol will be submitted to the Ethics Committee of the Faculty of Health Sciences, University of the Free State (UFS) for ethical consideration. Also, an approval to conduct the research will be obtained from the Free State Department of Health (DOH) after an ethical approval has been obtained as an ethics number is required for this application.

Patient's consent or assent will not be required as the study is a retrospective study and there will be no active participants.

However, all data collected will be anonymised, thereby maintaining patient's confidentiality. Number coding will be used to ensure the confidentiality of the infants. No names or personal identifiers will appear on any data sheet sent for statistical analysis.

13. Statistics

13.1. Pilot study

A pilot study will be conducted as soon as an approval from the Ethics Committee to conduct the research has been granted. The pilot study will be conducted on only 5 participants born out of the study period, to determine the feasibility of the study, to detect flaws in the protocol, test the suitability of the measurement instruments and to get an indication of possible results. Any shortfalls detected will allow for necessary changes on the data capture sheet prior to commencement of the research.

The data from the pilot study will not be included in the main research results.

13.2. Data capturing and analysis

All the data collected will be sent to the Department of Biostatistics of the UFS for analysis. All the results will be summarised as categorical variables (i.e. percentages) and numerical variables (i.e. mean, standard deviations, or percentiles).

14. Value of this study

There is no published research done on neonatal thrombocytopaenia in the Free State, and therefore no data currently exists to offer evidence-based advice on platelet transfusion guidelines in our practice.

This study will provide insights into the causative risk factors for neonatal thrombocytopaenia in the neonates admitted to the NHCU at PAH. This knowledge will help in the strategic planning regarding efforts made to reduce neonatal morbidity and mortality in the unit. Also, the information from this study will aid the clinicians in decision making regarding the role of platelet transfusions in preventing bleeding.

15. Limitations of the study

Owing to the nature of the study design and method of patient's clinical data collection, which will rely on the availability and completeness of the medical records, there is bound to be some limitations. Incomplete, and/or missing medical records will impact on the results of this study.

16. Time schedule for the execution of the study

Preliminary literature study	January - March 2018
Write protocol	April – May 2018
Submit to study leader/Evaluation Committee	May 2018
Submit final protocol to Biostatistics	May 2018
Submit to Ethics Committee	May – June 2018
Submit to Dept. of Health	June - July2018
Pilot study and Data collection	July – August 2018
Submit Data to Biostatistics	September 2018
Write thesis	October- November 2018
Revise thesis	December 2018
Printing, binding and submission of final thesis	December 2018

17. Budget

The budget of this study will be approximately R800 (eight hundred rand). This budget will cover all the expected costs for stationaries, printing and binding of the thesis and will be on the account of the researcher. The cost will be split as follows:

Papers/stationary	R150.00
Printing/binding	R500.00
Transport to and fro PAH (5.7km x2) at R11.2 per km	R150.00
Total:	R800.00

18. REFERENCES:

1. Carr R, Kelly AM, Williamson LM. Neonatal thrombocytopenia and platelet transfusion - A UK perspective. Neonatology. 2015;107(1):1–7.

2. Sparger K, Deschmann E, Sola-Visner M. Platelet Transfusions in the Neonatal Intensive Care Unit. Clin Perinatol. 2015;42(3):613–23.

3. Saini R, Saini P, Sehra RN, Saini L. Thrombocytopenia burden and its associating Risk factors: A cross-sectional study at a tertiary care set up. 2017;(7):237–43.

4. Tirupathi K, Swarnkar K, Vagha J. Study of risk factors of neonatal thrombocytopenia. Int J Contemp Pediatr. 2017;4(1):191–6.

5. Ayadi I.D, Ben Hamida E, Youssef A, Sdiri Y, Marrakchi Z. Prevalence and outcomes of thrombocytopenia in a neonatal intensive care unit. La Tunisie Medicale. 2016;94(4):305–8.

6. Kalagiri R, Choudhury S, Carder T, Govande V, Beeram M, Uddin M. Neonatal Thrombocytopenia as a Consequence of Maternal Preeclampsia. Am J Perinatol Reports [Internet]. 2015;6(1): e42–7. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0035-1565923.

7. Goyal P, Gupta S, Natani BS, Agarwal A, Bhatia S. Maternal and Neonatal Risk Factors for Thrombocytopenia in Preterm Infants. Indian Journal of Basic and Applied Medical Research 2017;6(2):39–46.

8. Fustolo-Gunnink SF, Vlug RD, Heckman EJ, Pas AB, Fijnvandraat K, Lopriore E. Early-onset thrombocytopenia in small-for-gestational-age neonates: A retrospective cohort study. PLoS One. 2016;11(5):1–11.

9. Boutaybi N, Razenberg F, Smits-Wintjens VEHJ, van Zwet EW, Rijken M, Steggerda SJ, et al. Neonatal Thrombocytopenia after Perinatal Asphyxia Treated with Hypothermia: A Retrospective Case Control Study. Int J Pediatr [Internet]. 2014; 2014:1–6. Available from: http://www.hindawi.com/journals/ijpedi/2014/760654/.

10. Eslami Z, Lookzadeh MH, Noorishadkam M, Hashemi A, Ghilian R, Pirdehghan A. Thrombocytopenia and Associated Factors in Neonates Admitted to NICU during Years 2010_2011. Iran J Pediatr Hematol Oncol [Internet]. 2013;3(1):205–15. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3915438&tool=pmcentrez&rendertype=abstract.

11. Iqbal Q, Bashir C, Mushtaq S, Ahmad A, Baba AR. Thrombocytopenia and other hematological parameters in culture positive neonatal sepsis and their impact. J Pediatr Infect Dis.

2013;8(1):25–9.

12. Ulusoy E, Tüfekçi Ö, Duman N, Kumral A, Irken G, Ören H. Thrombocytopenia in neonates: Causes and outcomes. Ann Hematol. 2013;92(7):961–7.

13. Ree IMC, Fustolo-Gunnink SF, Bekker V, Fijnvandraat KJ, Steggerda SJ, Lopriore E. Thrombocytopenia in neonatal sepsis: Incidence, severity and risk factors. PLoS One. 2017;12(10):1–11.

14. Cremer M, Sallmon H, Kling PJ, Bührer C, Dame C. Thrombocytopenia and platelet transfusion in the neonate. Semin Fetal Neonatal Med [Internet]. 2016;21(1):10–8. Available from: http://dx.doi.org/10.1016/j.siny.2015.11.001.

15. Sparger KA, Assmann SF, Granger S, Winston A, Christensen RD, Widness JA, et al. Platelet transfusion practices among very-low-birth-weight infants. JAMA Pediatr. 2016;170(7):687–94.5.

16. Sola-Visner M, Bercovitz RS. Neonatal Platelet Transfusions and Future Areas of Research. Transfus Med Rev [Internet]. 2016;30(4):183–8. Available from: http://dx.doi.org/10.1016/j.tmrv.2016.05.009.

17. Boutaybi N, Steggerda SJ, Smits-Wintjens VEHJ, van Zwet EW, Walther FJ, Lopriore E. Early-onset thrombocytopenia in near-term and term infants with perinatal asphyxia. Vox Sang. 2014;106(4):361–7.

18. Bolat F, Kiliç SÇ, Oflaz MB, Gülhan E, Kaya A, Güven AS, et al. The prevalence and outcomes of thrombocytopenia in a neonatal intensive care unit: A three-year report. Pediatr Hematol Oncol. 2012;29(8):710–20.

19. Tiller H, Husebekk A, Ahlen MT, Stuge TB, Skogen B. Current perspectives on fetal and neonatal alloimmune thrombocytopenia - Increasing clinical concerns and new treatment opportunities. Int J Womens Health. 2017; 9:223–34.

20. Yurdakök M. Immune thrombocytopenia in the newborn. 2017;6(1):1–10.

21. Chakravorty S, Roberts I. How I manage neonatal thrombocytopenia. Br J Haematol. 2012;156(2):155–62.

22. Holzhauer S, Zieger B. Diagnosis and management of neonatal thrombocytopenia. Semin Fetal Neonatal Med [Internet]. 2011;16(6):305–10. Available from: http://dx.doi.org/10.1016/j.siny.2011.07.008.

23. Joolay, Yaseen; Horn, Alan; Raban, M. Shukri; Harrison, Michael; Tooke, Lloyd; Rhoda N. Neonatal guidelines and drug doses. 1st ed. Joolay, Yaseen; Horn A, editor. Cape Town: The

Science Press; 2012. 78 p.

24. Wittenberg D. Coovadia's paediatrics & child health. 6th ed. Coovadia H, editor. Cape Town: Oxford University Press Southern Africa (Pty) Ltd; 2009. 120 p.

25. Cloherty, John P; Eichenwald, Eric C; Hansen, Anne R; Stark AR. Manual of Neonatal Care. 7th ed. Cloherty, John P; Eichenwald, Eric C; Hansen, Anne R; Stark AR, editor. Philadelphia: Lippincott Williams & Wilkins; 2012. 154 p.

26. Gomella, Tricia Lacy; Cunningham, M. Douglas; Eyal FG. NEONATALOGY Management, Procedures, On-Call Problems, Diseases, and Drugs. 7th ed. Gomella, Tricia Lacy; Cunningham, M. Douglas; Eyal FG, editor. New York: McGraw Hill Education LLC; 2013. 154 p.

19. Appendix:

Appendix A: Data Capture Sheet:

DATA CAPTURE SHEET						
Neonatal Factors						
Infant's Case Number						
Date/Time of Birth	Dd	mm	yy	/ ……h……..		
Date/Time of Admission	Dd	mm	yy	/…….h……..		
Gender		Female=0		Male = 1		
Birth Weight		0=≥2500g		3 = 1000-1249g		
		1 = 1500-2499g		4 = 800-999g		
		2 = 1250-1499g		5 = <800g		
Gestational Age		0=>37wks		2=30-31wks		
		1=32-37wks		3=28-29wks	4=<28wks	
Appropriateness for Gestational Age		0 = SGA	1 = AGA	2 = LGA		
Congenital Abnormality		0 = No	1 = Yes			
			If yes: Specify: ----------			
Further workup done: e.g. peripheral blood smears, head sonar		0 = No	1 = Yes			
Birth Asphyxia		0 = No	1 = Yes			
History of fetal distress on CTG: Y/N						
Apgar scores at 5min:						
Blood gas Base Excess						
Resp failure requiring resus during 5min after birth: Y/N						

Multiple organ failure: Y/N		
Sepsis at time of Thrombocytopaenia		0 = No 1 = Yes
TORCH Infection		0 = No 1 = Yes 2 = Not done
Platelet count		0 = <10 2 = 20-29 4 = 50-99 1 = 10-19 3 = 30-49 5 = 100-149
Timing of the Thrombocytopaenia		0 = Early-onset (<72h) 1 = Late-onset (≥72h)
Platelet Transfusion		0 = No 1 = Yes
If Yes: Platelet Transfusion Repeated		0 = x 2 1 = > x 2
IVH		0 = No 1 = Yes 2 = Not done
NEC		0 = No 1 = Yes
OUTCOME: Discharge/Demise		1 = Discharge 0=Demise

-

Maternal/Perinatal Factors		
APH		0 = No 1 = Yes
PIH		0 = No 1 = Yes
PET/Eclampsia		0 = No 1 = Yes
HELLP Syndrome		0 = No 1 = Yes
GDM		0 = No 1 = Yes
ITP		0 = No 1 = Yes
PROM		0 = No 1 = Yes

Chorioamnionitis	☐ 0 = No 1 = Yes

3.5 APPENDIX E: Data collection form:

DATA CAPTURE SHEET				
Neonatal Factors				
Infant's Case Number				
Date/Time of Birth	Dd	mm	yy	/ ……h…….
Date/Time of Admission	Dd	mm	yy	/…….h……..
Gender		Female=0		Male = 1
Birth Weight		0 ≥ 2500g 1 = 1500 - 2499g 2 = 1250 – 1499g		3 = 1000 - 1249g 4=800-999g 5 = <800g
Gestational Age		0=>37wks 1=32-37wks		2=30-31wks 3=28-29wks 4=<28wks
Appropriateness for Gestational Age		0 = SGA	1 = AGA	2 = LGA
Congenital Abnormality		0 = No	1 = Yes If yes: Specify: ----------	
Further workup done: e.g. peripheral blood smears, head sonar		0 = No	1 = Yes	
Birth Asphyxia		0 = No	1 = Yes	
History of fetal distress on CTG: Y/N				
Apgar scores at 5min:				
Blood gas Base Excess				
Resp failure requiring resus during 5min after birth: Y/N				

Multiple organ failure: Y/N		
Sepsis at time of Thrombocytopaenia		0 = No 1 = Yes
TORCH Infection		0 = No 1 = Yes 2 = Not done
Platelet count		0 = <10 1 = 10-19 2 = 20-29 3 = 30-49 4 = 50-99 5 = 100-149
Timing of the Thrombocytopaenia		0 = Early-onset (<72h) 1 = Late-onset (≥72h)
Platelet Transfusion		0 = No 1 = Yes
If Yes: Platelet Transfusion Repeated		0 = x 2 1 = > x 2
IVH		0 = No 1 = Yes 2 = Not done
NEC		0 = No 1 = Yes
OUTCOME: Discharge/Demise		1 = Discharge 0 = Demise

-

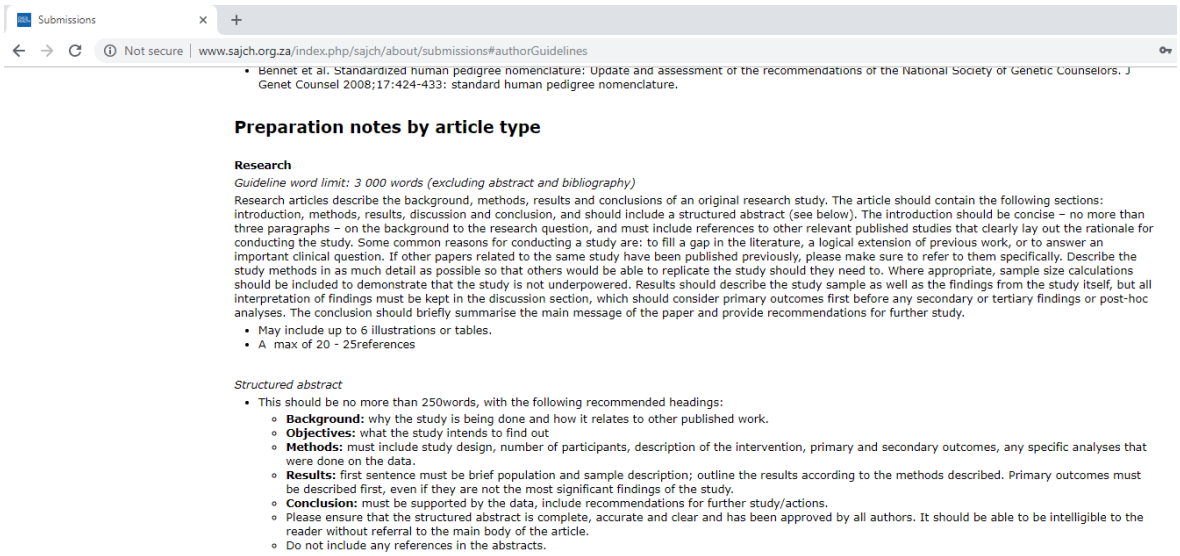
Maternal/Perinatal Factors		
APH		0 = No 1 = Yes
PIH		0 = No 1 = Yes
PET/Eclampsia		0 = No 1 = Yes
HELLP Syndrome		0 = No 1 = Yes
GDM		0 = No 1 = Yes
ITP		0 = No 1 = Yes
PROM		0 = No 1 = Yes

Chorioamnionitis		0 = No 1 = Yes

3.6 APPENDIX F: Instructions to authors of the journal:

South African Journal of Child Health

http://www.sajch.org.za


Submissions

Not secure | www.sajch.org.za/index.php/sajch/about/submissions#authorGuidelines

- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Guideline word limit: 3 000 words (excluding abstract and bibliography)

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Where appropriate, sample size calculations should be included to demonstrate that the study is not underpowered. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

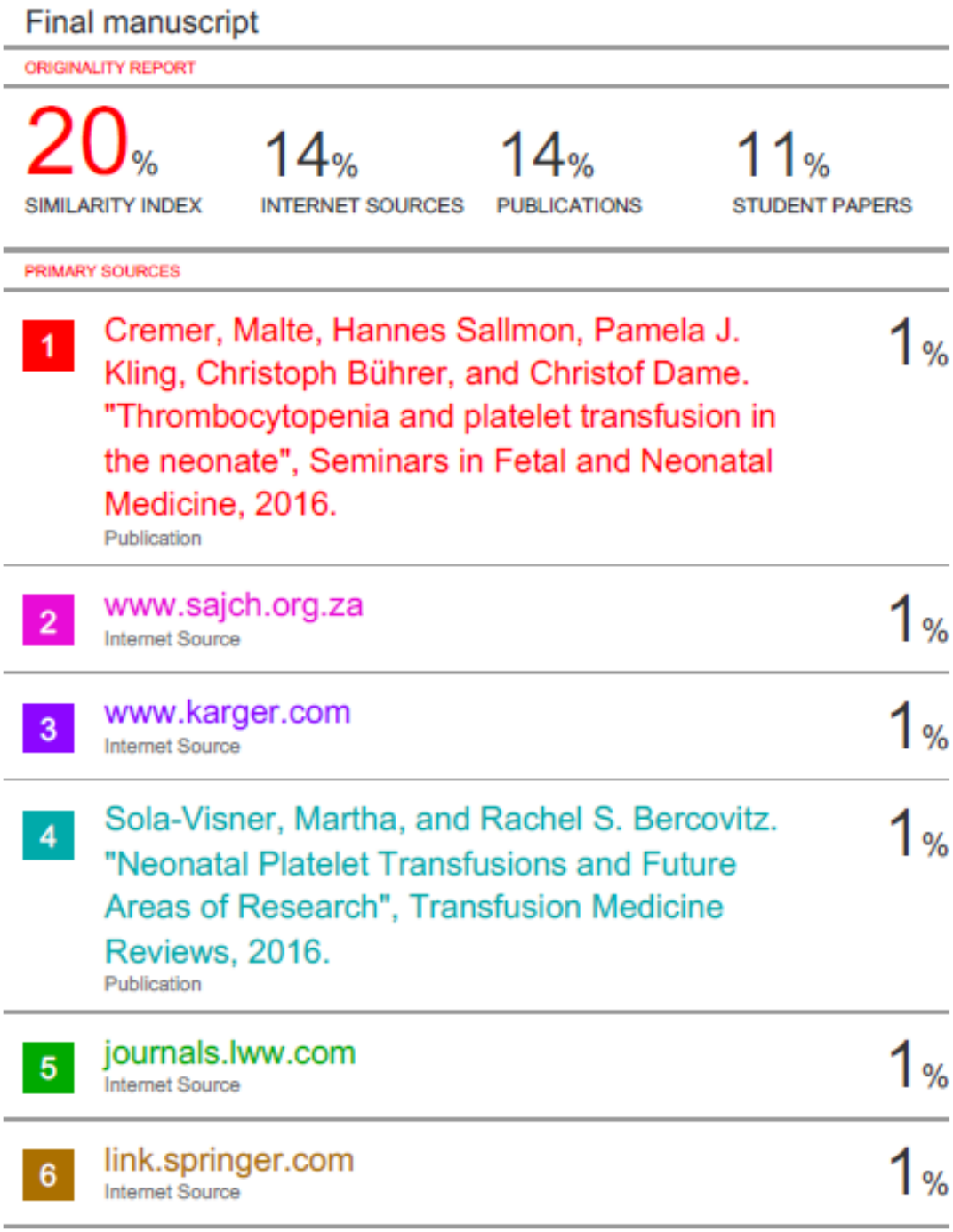
- May include up to 6 illustrations or tables.
- A max of 20 - 25references

Structured abstract

- This should be no more than 250words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
  - Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors. It should be able to be intelligible to the reader without referral to the main body of the article.
  - Do not include any references in the abstracts.

Source: http://www.sajch.org.za/index.php/sajch/about/submissions#authorGuidelines

3.7 APPENDIX G: A summary report compiled in the Turnitin Plagiarism Search Engine:



Final manuscript

ORIGINALITY REPORT

20%	14%	14%	11%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Cremer, Malte, Hannes Sallmon, Pamela J. Kling, Christoph Bührer, and Christof Dame. "Thrombocytopenia and platelet transfusion in the neonate", Seminars in Fetal and Neonatal Medicine, 2016. Publication	1%
2	www.sajch.org.za Internet Source	1%
3	www.karger.com Internet Source	1%
4	Sola-Visner, Martha, and Rachel S. Bercovitz. "Neonatal Platelet Transfusions and Future Areas of Research", Transfusion Medicine Reviews, 2016. Publication	1%
5	journals.lww.com Internet Source	1%
6	link.springer.com Internet Source	1%