Thrombocytopenia in Healthy Term Infants: Incidence and Clinical outcomes at Middlemore Hospital, New Zealand.

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Abstract

Background

Neonatal alloimmune thrombocytopenia (NAIT) is caused by the destruction of an infant's platelets by maternal allo-antibodies developed in utero against paternally acquired antigens on the foetus' platelets. It can be associated with considerable morbidity and mortality. The incidence of NAIT is unknown in New Zealand and therefore considered uncommon and probably under-diagnosed.

This study was initially designed to determine the incidence of NAIT in infants born at Middlemore Hospital, in a period of 11 years. However, it was not possible to determine the incidence from the beginning considering the definition of incidence therefore prevalence was used instead. The objectives of the study are to determine the prevalence of thrombocytopenia in otherwise healthy term infants of varying ethnicities; investigate associations of thrombocytopenic infants with clinical outcomes in affected infants and subsequent siblings and develop a suggested guideline for future detection, management of NAIT in New Zealand and Identify cases of NAIT in asymptomatic infants.

This retrospective study involved analysis of 66910 cord blood full blood count (FBC) results from babies born at Middlemore Hospital between 2005 and mid – 2016. All babies with a platelet count below the reference range ($150x\ 10^9/L$) were selected. Relevant clinical data were collected from the clinical records of these babies and mothers.

Of the total number of cord blood FBC analysed, 1.2% showed thrombocytopenia. Of these, 80.7% were term babies and the rest were preterm. Amongst these thrombocytopenic infants, 67% had no abnormal clinical features suggestive of thrombocytopenia. These asymptomatic infants were mostly term babies, the majority of whom were not followed up with repeat counts, except one, who returned with symptomatic thrombocytopenia 5 days after discharge. This infant was later diagnosed with NAIT. Investigations of family history of almost all asymptomatic thrombocytopenic infants (n = 532) examined reported that 1.9% of the mothers had one or more stillbirths, and 20.5% had experienced miscarriages. None of these mothers however, appeared to have had any association with NAIT, nor cases of intracranial haemorrhage (ICH). Furthermore, while sixteen infants (4.9%) had siblings with documented thrombocytopenia, none were attributed to NAIT.

One third of the infants who were thrombocytopenic at birth were admitted for a variety of medical indications. Amongst these indications, 10 cases (0.0006% of the total number of cord blood FBC) were attributed to NAIT, of which only 3 were confirmed serologically. It can be concluded from this study that NAIT is quite uncommon in this setting despite the large number of term infants born with thrombocytopenia. It is therefore suggested that the cut off range for thrombocytopenia in cord blood in this setting must be reviewed.

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Contents

List of Figures

List of Tables

Attestation of authorship

“I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.”

Signature _____

Date_____

List of Abbreviations

ADHB	Auckland District Health Board
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AUT	Auckland University of Technology
bFGF	Basic Fibroblast Growth Factor
BW	Birth Weight
CI	Confidence Interval
CMDHB	Counties Manukau District Health Board
CMV	Cytomegalovirus
DIC	Disseminated Intravascular Coagulation
EGF	Epidermal Growth Factor
FBC	Full Blood Count
FC – PIFT	Flow Cytometry Platelet Immunofluoresence Test
FGF	Fibroblast Growth Factor
FHES	Faculty of Health and Environmental Sciences
FMAIT	Foeto – maternal Alloimmune Thrombocytopenia
FNAIT	Foetal/neonatal Alloimmune Thrombocytopenia
GDM	Gestational Diabetes Mellitus
GM – CSF	Granulocyte Macrophage Colony Stimulating Factor
GP	Glycoprotein
HDN	Haemolytic Diseases of the Newborn

HIE	Hypoxic Ischaemic Encephalopathy
HLA	Human Leukocyte Antigen
HPA	Human Platelet Antigen
HUS	Haemolytic Uraemic Syndrome
HW	Healthware
ICH	Intracranial Haemorrhage
IL	Interleukin
ITP	Idiopathic Thrombocytopenic Purpura
IUGR	Intrauterine Growth Restrictions
IVH	Intraventricular Haemorrhage
IVIG	Intravenous Immunoglobulin
LBW	Low Birth Weight
LGA	Larger than Gestation Age
MCV	Mean Cell Volume
MHC	Major Histocompatibility Complex
MGDF	Megakaryocyte Growth and Development factor
NAIT	Neonatal alloimmune thrombocytopenia
NHI	National Health Index
NICU	Neonatal Intensive Care Unit
NND	Neonatal Death
NZBS	New Zealand Blood Service
NZMFMN	New Zealand Maternal Foetal Medicine Network

PDA	Patent Ductus Arteriosus
PET	Preeclamptic Toxaemia
PPHN	Persistent Pulmonary Hypertension of the Newborn
PTP	Post Transfusion Purpura
RDS	Respiratory Distress Syndrome
RR	Relative Risk
RT – PCR	Real Time Polymerise Chain Reaction
SDF	Stromal Cell – Derived Factor
SGA	Smaller than Gestation Age
SLE	Systemic Lupus Erythromatous
SPA	Serum Platelet Antibodies
TNF	Tumour Necrosis Factor
TPO/THPO	Thrombopoietin
TOP	Termination of Pregnancy
TORCH	Toxoplasma gondii, other viruses (HIV, Measles and so on), Rubella (German Measles), Cytomegalovirus, Herpes Simplex
TTN	Transient Tachypnoea of Newborn
TTP	Thrombotic Thrombocytopenic Purpura
UGA	Unknown Gestation Age
VCA	Vulnerable Children's Act
VEGF	Vascular Endothelial Growth Factor
VSD	Ventricular Septal Defect
vWF	von Willebrand factor

Chapter 1 Platelets

1.1 Introduction

Platelets are tiny cell fragments that are produced in the bone marrow from the cytoplasm of megakaryocytes. They play a significant role in haemostasis (Machlus & Italiano, 2013).

This chapter reviews current understanding of platelet biology and thrombocytopenia. The discussion includes aspects of platelet production, structure and function, and an overview of when there is a quantitative reduction in platelet numbers known as thrombocytopenia. The known causes of thrombocytopenia are reviewed and discussed.

1.2 Platelet biology

1.2.1 Platelet production

Megakaryocytes arise from haemopoietic stem cells and are the biggest myeloid cells in the bone marrow (Machlus & Italiano, 2013; Masamoto & Kurokawa, 2016; Patel, Hartwig, & Italiano, 2005). They have a shape of about 50 – 100 μm in diameter and comprise less than 1% of nucleated bone marrow cells. Apart from their primary location in the bone marrow, they may also be found in the lungs and rarely the peripheral blood. At different stages of development from embryo to adult, they are produced by the yolk sac, liver, spleen, and bone marrow (Machlus, Thon, & Italiano, 2014).

The earliest recognisable cell specific to platelet production is the megakaryoblast. This differentiates into the promegakaryocyte, which further differentiates into the granular megakaryocyte. Finally, platelets are produced by fragmentation of megakaryocyte cytoplasm (Guo et al., 2015; X. R. Xu, Zhang, et al., 2016) (Figure 1.1). They then extruded from the parent cell into small vascular sinusoids in the bone marrow and enter into circulation.

Unique amongst mammalian cells, megakaryocytes undergo endomitosis (DNA replication without cell division) whereby they become polypoid as they mature into

cells of ever-increasing size. During this process, much of their cytoplasm is enclosed in an extensive canalicular system; these cytoplasmic "packages" are called proplatelets as they are precursors to platelets (Italiano, 2017; Kaushansky, 2008). After platelets are released from bone marrow, they are sequestered in the spleen for one to two days, and then re-enter the circulation. Their normal life span is about 7 to 10 days (Sillers, Van Slambrouck, & Lapping-Carr, 2015) and approximately 1 x 10^{11} platelets are made per day (Machlus & Italiano, 2013).

The maturation process and development of megakaryocytes requires specific growth factors including granulocyte – macrophage colony – stimulating factor (GM – CSF), interleukin (IL – 3), IL – 6, IL – 11, chemokines (stromal cell-derived factor – 1 (SDF – 1), fibroblast growth factors – 4 (FGF – 4) to maintain a constant platelet production (Machlus & Italiano, 2013).

Platelet production (thrombopoiesis) is regulated by the glycoprotein hormone thrombopoietin (THPO) (Bianchi, Norfo, Pennucci, Zini, & Manfredini, 2016), which is also known as megakaryocyte growth and development factor (MGDF). It is a glycoprotein hormone encoded by THPO gene and primarily produced in the liver and kidney, and in small amounts in the bone marrow (Guo et al., 2015). Thrombocytes have receptors to thrombopoietin called c-Mpl receptors which serve as medium in autoregulation of platelet production (Kaushansky, 2005). For example, during thrombopoiesis, as platelet levels rise, c-Mpl ligands on haemopoietin bind avidly to c-Mpl platelet receptors and thus lowering the level of free functional platelets. This then creates a negative feedback effect on thrombopoietin and hence synthesis of platelets ceases (Moreau et al., 2016). During thrombocytopenia on the other hand, there are fewer platelets to which c-Mpl ligands can attach and therefore this generates a positive feedback effect on thrombopoietin release. Its release thus promotes thrombopoiesis (Kaushansky, 2005; Momi & Wiwanitkit, 2017).

During an acute loss of platelets~~,~~ thrombopoietin production is increased as a result of positive feedback regulation that results in increased thrombopoiesis to compensate for increased loss (Masamoto & Kurokawa, 2016).

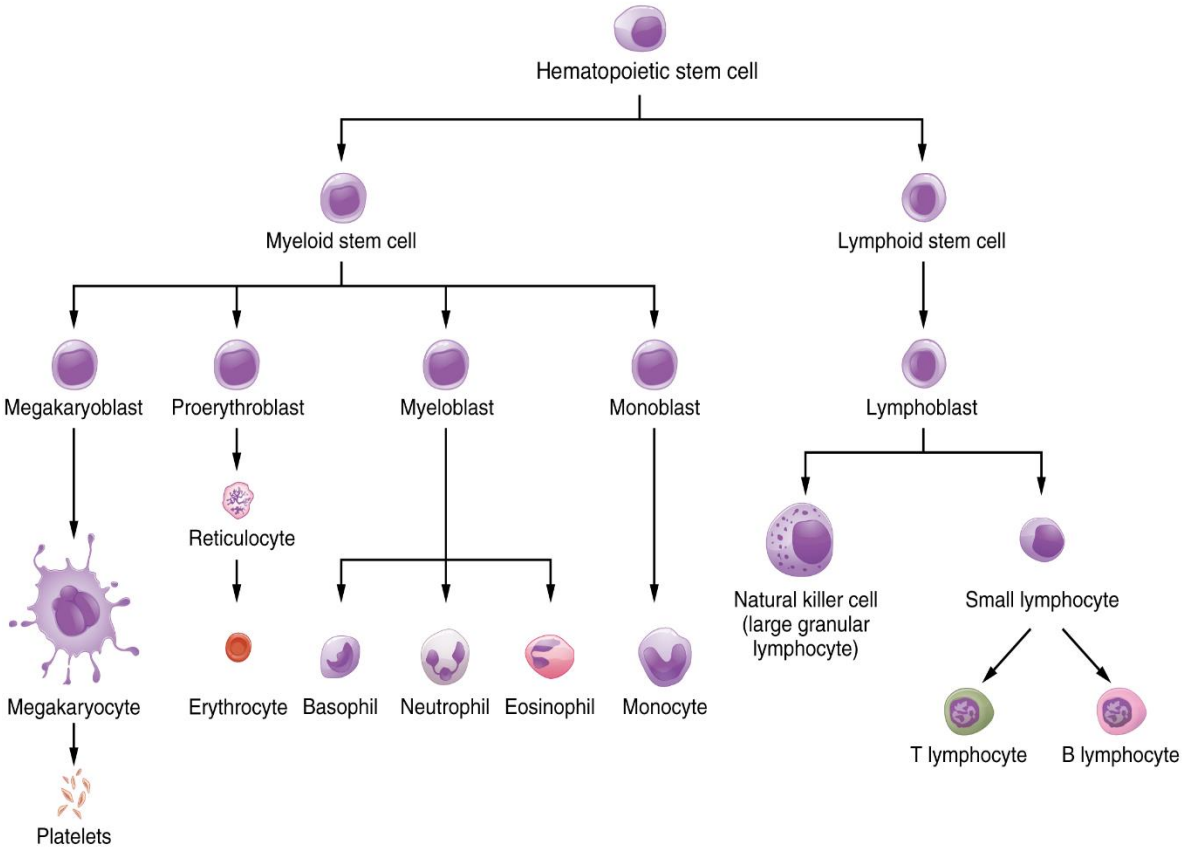

Figure 1.1. Hierarchy in haemopoietic cells. Proliferation and differentiation of blood cells.

1.2.2 Platelet Structure

Platelets are small, non - nucleated blood cells produced from fragmentation of the cytoplasm of megakaryocytes in the bone marrow (Vadasz et al., 2015). They have a biconvex discoid shape of about 2 – 3µm in greatest diameter and comprise four components: plasma membrane, sub membrane area, cytoskeleton (Sol-gel zone), and organelles (Fritsma, 2015) (Figure 1.2). The platelet membrane is a lipid bilayer and is partially or completely infiltrated by a variety of glycoprotein molecules which function as receptors for a variety of different agonists, adhesion proteins, coagulation factors, and other platelets (Zdravic et al., 2016).

The organelles comprise of lysosomes, peroxisomes, mitochondria, alpha (α) granules, and dense bodies. The alpha granules contain many of the coagulation factors such as ɬfactor V, XI, fibrinogen, and von Willebrand factor (vWF). It also contain many growth factors and some adhesion molecules, while dense bodies contain adenosine

diphosphate (ADP), adenosine triphosphate (ATP), serotonin, and calcium which are needed for successful coagulation (Geddis, 2010).

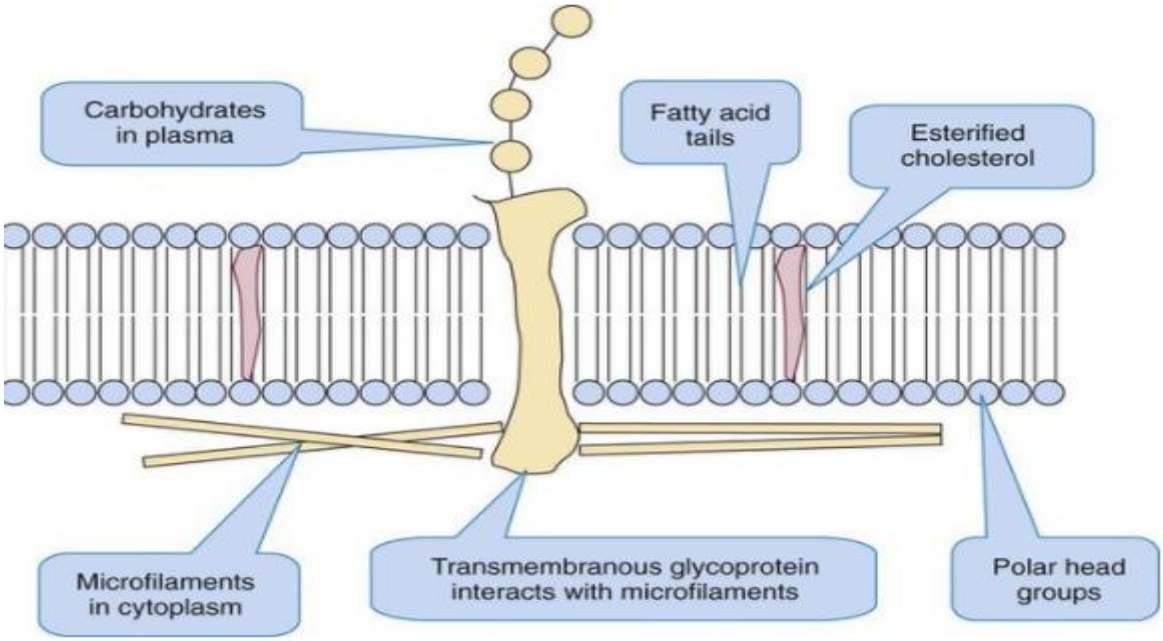

Figure 1.2. Diagram of platelet membrane structure. The platelet possesses a standard biologic membrane composed of a phospholipid bilayer, esterified cholesterol, and a series of transmembranous proteins.

1.2.3 Platelet Function

Platelets play a vital role in haemostasis, inflammation and immunity for both innate and adaptive immune responses (Sonneveld et al., 2016; Veldhuisen, Porcelijn, Ellen van der Schoot, & de Haas, 2014). During an injury to the endothelial lining of a blood vessel, platelets migrate to the site and form a haemostatic plug. They first adhere to the exposed sub endothelial matrix proteins via glycoprotein (GP) IIb/IIIa receptor complex (Curtis, 2015). Following activation, a sequence of events ensues almost simultaneously, and platelets release the contents of their various granules such as alpha, dense, and lysosomal. This secretory phase results in the stimulation of coagulation, increased vascular tone and permeability, and support of endothelial repair and wound healing. Platelets also adhere directly or indirectly via von Willebrand factor (vWF) to the exposed sub endothelial matrix, secrete their contents such as coagulation factors, and aggregate by linking together via fibrinogen bridges to form the platelet plug to stop blood loss through the wound (Wolber & Jelkmann, 2002).

1.3 Platelet glycoproteins

Several glycoproteins (GP) which are expressed on the platelet plasma membrane are essential in the interaction of platelets with sub endothelial connective tissue and other platelets, and play a vital role in platelet adhesion and aggregation (Hughes-Jones, 2008). These include GPIa, GPIb, and GPIIb/GPIIIa; each has a specific role: GPIa facilitates platelet binding to collagen, GPIb to von Willebrand factor (vWF), and GPIIb/GPIIIa to fibrinogen (Hughes-Jones, 2008).

1.4 Platelet antigens

Platelets express three major antigen systems including human leukocyte antigen (HLA) Class I antigen, ABO antigens and human platelet antigen (HPA) which are associated with glycoproteins (Bussel & Primiani, 2008). Studies have postulated that antibodies to these antigens can cause refractoriness to platelet transfusion, neonatal alloimmune thrombocytopenia (NAIT), and idiopathic thrombocytopenic purpura (Hayashi, Amakishi, Inoue, & Hirayama, 2011). Thus, the detection of these antibodies is important for diagnostic purposes.

1.4.1 ABO Antigens

Platelet glycoproteins express ABO antigens (Peterson, McFarland, Curtis, & Aster, 2013), especially on GPIIb and the platelet endothelial cell adhesion molecule (PECAM – 1, CD31). These authors state that healthy individuals with blood groups A and B have platelets that express remarkably large numbers of A and B antigen sites. The variations in these antigenic sites vary between the different populations in this study population (Peterson et al., 2013).

Moreover, Ahlen et al. (2012) found a correlation between maternal ABO phenotype and genotype and the severity of thrombocytopenia of the newborn infant.

1.4.2 Human Leucocyte Antigen

The human version of the major histocompatibility complex (MHC) is human leucocyte antigen (HLA) which is located on the short arm of chromosome 6 (Choo, 2007). It is a group of highly polymorphic genes or cell surface antigens that present antigens to T cells as an immune response to infection (Solberg et al., 2008; Tshabalala, Mellet, & Pepper, 2015). HLAs are all expressed on almost all the cells in the body. These antigens primarily function to serve as recognising molecules in the initiation of an immune response by presenting antigens from foreign substances, e.g., viruses and bacteria, to effector cells of the immune system, that is, by assisting the immune system to differentiate the body's own proteins from foreign proteins made by viruses and bacteria. HLAs are also responsible for the regulation of the immune system in humans (Tshabalala et al., 2015).

HLA comprise three (3) classes of antigens; HLA class I, class II and class III. Class I encodes 3 main loci; HLA – A, - B, and – C; HLA class II encodes three main loci HLA – DP, - DQ, and – DR; and HLA class III is referred to as complement region located between class I & class II regions (Hutchinson, Dennington, Holdsworth, & Downe, 2015).

The HLA class I and II antigens are cell – surface glycoproteins encoded to bind intra – and extracellular antigens respectively, whereas HLA Class III genes are normally encoded with various secreted proteins that play a role in immune responses comprising components of the complement system (C2, C4, BF) and molecules involved in inflammation (tumour necrosis factor - TNF, & heat-shock proteins) (Hershfield et al., 2013; Mosaad, 2015). Studies demonstrate that HLA Class I antigens are expressed on all blood cells in the body, and encoded by genes which are highly polymorphic in the human genome (Bonstein & Haddad, 2017; Hoffbrand, Catovsky, & Tuddenham, 2005).

Incompatibility in HLA Class I antigens can initiate the host immune system to produce alloantibodies. Consequently, the presence of anti-HLA class I antibodies can result in serious situations such as the rejection of allografts or the destruction of transfused platelets (Dahl et al., 2016). Incompatibility of HLA Class I antibodies have also been

implicated in a variety of pregnancy complications including recurrent miscarriage, placental abruption, and preeclampsia (Dahl et al., 2016).

1.4.3 Human Platelet Antigens.

Platelets express a large number of genes coding for human platelet antigens (HPA). These are biallelic amino acid polymorphisms with various nucleotide substitutions and are inherited in an autosomal co-dominant manner (Mella & Eddleman, 2015; Veldhuisen et al., 2014) (Figure 1.3).

These HPA antigens are expressed on platelet membrane glycoproteins (GPs) GPIb – V – IX (von Willebrand receptor), GPIIb/IIIa (αIIb/β3 integrin, fibrinogen receptor), GPIa/IIa (a collagen receptor) and CD109, a glycosylphosphatidylinositol (GPI) – anchored protein of uncertain function (Peterson et al., 2013; Weng et al., 2016). HPAs are reported to be involved in the pathogenesis of several clinical conditions, including neonatal alloimmune thrombocytopenia (NAIT), platelet transfusion purpura (PTP) and refractoriness to platelet transfusion (Al-Ouda, Al-Banyan, Abdel Gader, Bayoumy, & Al-Gahtani, 2016).

To date, more than thirty – five HPAs have been reported since the first description in 1960s (Curtis, 2015). They are expressed on six different platelet glycoproteins, GPIIb (CD41), GPIIIa (CD61), GPIb (CD42), GPIbβ (CD42c), GPIa (CD49b), and CD109 (Guz et al., 2017). For the current list, visit http://www.ebi.ac.uk/ipd/hpa/table1.html. GPIIIa express most of the HPA as this involves GPIIb/IIIa (integrin $\alpha_2 b\beta_3$) complex, which is the most important receptor of human platelets for fibrinogen, vitronectin, fibronectin, and von Willebrand factor. Endothelial cells also express GPIIIa as part of αVβ3 integrin, which is the receptor for vitronectin and other proteins (Guz et al., 2017).

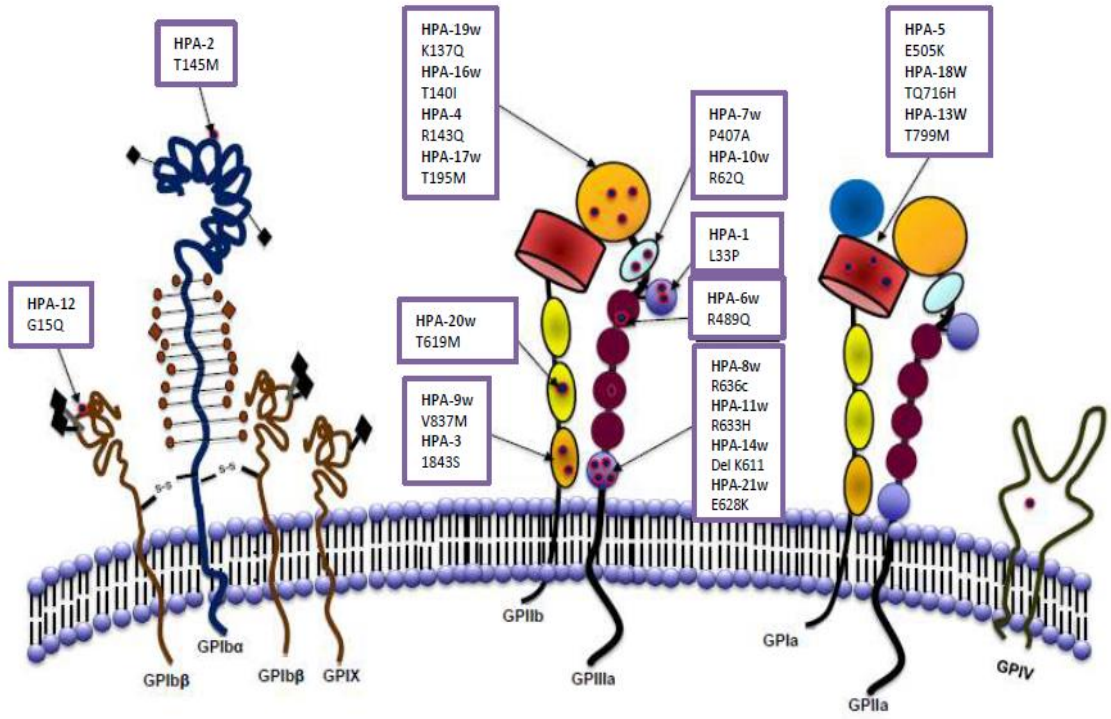

Figure 1.3. This figure illustrates some of the major Human Platelet Antigens (HPAs) on some of the various glycoprotein complexes which are responsible for alloimmunizing pregnant women leading to FNAIT. Most HPA variants? are located on the integrin β3 subunit and are due to a single amino acid substitution; an exception being HPA-14w which results from a one amino acid deletion.

The frequency of HPA varies with different ethnic population. In Caucasian population, the most common HPAs are HPA – 1a and HPA – 5b, in Japanese and Asians populations are HPA – 2b, - 5b and – 6b, in Maori are HPA – 2b and – 3a, and in Polynesians is HPA – 6b. Other ethnic populations such as Middle East, Africans and Americans are reported to exhibit antigens that can implicate diseases such as NAIT and platelet refractoriness (Londero, Miani, Rinaldi, Totis, & de Angelis, 2018; Silva et al., 2011).

1.5 Thrombocytopenia

Thrombocytopenia refers to a platelet count below the reference range for age and population. There is uncertainty about the actual reference range for platelet count in low birth weight infants. Low birth weight infants include those born less than 32 weeks of gestation, late preterm infants and healthy term infants and weighing 1,000g

(Yurdakok, 2017). Ideally, each laboratory should produce its own reference ranges to suit its context. Although controversial, the majority of laboratories use the adult reference range of 150 to 400 x 10^9/L for healthy foetuses (mid-second trimester) and neonates (Wong & Glader, 2004).

It is reported that thrombocytopenia is relatively common in preterm infants than term, and that it may be experienced a few days after birth but improve within one week (Sillers et al., 2015) (Roberts, Stanworth, & Murray, 2008).

Thrombocytopenia is classified as mild when the platelet count is in the range of 100 - 149 x 10^9/L, moderate when in the range of 50 – 99 x 10^9/L, and severe when platelets are below 50 x 10^9/L (Cremer, Sallmon, Kling, Buhrer, & Dame, 2016). It is an indication that platelets are being removed from the circulation faster than they are being replaced. This can lead to ineffective clot formation and increases the risk of bleeding or decreased platelet production. (G. Bertrand, Leguen, Delugin, & Renac, 2017; Patel et al., 2005).

Clinical symptoms are only likely to occur when there is severe thrombocytopenia (van den Akker & Oepkes, 2008).

The effects of low platelet count and/or impaired function are; increased vascular fragility and purpura, i.e., bleeding into skin and mucous membranes, and extensive haemorrhage after cuts (Nesbitt, Waxman, Cruz, Reyes-Taucher, & Sharathkumar, 2009).

1.5.1 Causes of thrombocytopenia

Thrombocytopenia is mainly due to decreased production or increased destruction of platelets, and/or abnormal platelet sequestration, as might occur with splenomegaly (Yurdakok, 2017). In neonates, thrombocytopenia is usually due to either increased consumption or decreased production. Depending on the cause, it is further categorised into three stages by time of onset. These stages are; foetal thrombocytopenia (if already present at birth); early onset (if detected soon after or developing within 72 hours of birth) and late onset (if it develops after the immediate postnatal period) (Becocci et al., 2018; Carr, Watts, & Rea, 2017). An alternative classification is whether it is hereditary

or acquired, in which case the causes may be immune mediated or non – immune. (Morrone, 2018; Peters & Grainger, 2017; Vadasz et al., 2015).

Thrombocytopenia in most infants can be explained by non – immune causes (Carr et al., 2017) such as prematurity, sepsis, disseminated intravascular coagulation [DIC], asphyxia, chromosomal abnormalities, maternal illnesses, and rarely metabolic or hereditary platelet disorders (Risson, Davies, & Williams, 2012). On the contrary, thrombocytopenia is rarely associated with specific immune causes such as neonatal alloimmune thrombocytopenia, thrombocytopenia due to maternal autoimmune thrombocytopenia (ITP) and hereditary thrombocytopenia's (Carr et al., 2017).

1.5.1.1 Immune

Immune mediated neonatal thrombocytopenia is classified as autoimmune or alloimmune mediated (Gunnink et al., 2014; Zdravic et al., 2016).

Autoimmune thrombocytopenia

Autoimmune mediated thrombocytopenia occurs as a result of an abnormal immune response in which the immune system of patients develop antibodies that react with a person's own tissues or organs, targeting various glycoproteins on the surface of platelets (Refsum, Meinke, Gryfelt, Wikman, & Höglund, 2018; Zdravic et al., 2016).

Autoimmune mediated thrombocytopenia is further classified into primary immune thrombocytopenia (ITP) and secondary causes such as infection or drug – induced. The most common type is primary immune thrombocytopenia (ITP) which accounts for 1 – 2 per 10, 000 people (Eyada, Amin, Samih, & Khedr, 2018; James, 2018; Zdravic et al., 2016), although the exact mechanism is not fully understood. Studies have implicated that increased platelet destruction, increased sequestration in organs such as the spleen, and decreased platelet production all contribute to primary ITP. Clinical symptoms range from mild to severe and potentially life threatening (Kistanguri & McCrae, 2013).

Alloimmune thrombocytopenia

Alloimmune thrombocytopenia is triggered by anti – platelet glycoprotein antibodies, which are produced by the host patient's immune response after being exposed to

foreign – or allogeneic platelets (Meler, Porta, Canals, Serra, & Lozano, 2017; Zdravic et al., 2016). Thrombocytopenia may develop following transfusion of allogeneic donor platelets and is known as post transfusion purpura (PTP) (Guz et al., 2017; X. Xu & Santoso, 2018). Alternately it may arise during pregnancy when the mother's circulation is exposed to paternally derived human platelet antigens (HPA) on the surface of foetal platelets (Sonneveld et al., 2016).

Neonatal alloimmune thrombocytopenia (NAIT) is a type of alloimmune thrombocytopenia in which the mother's immune system generates alloantibodies targeting foetal platelets (Sainio, Javela, Tuimala, & Haimila, 2017). Studies have shown that the majority of NAIT cases arise in primigravidous pregnancies thus making proper diagnosis and treatment of FNAIT particularly difficult as no routine screening is performed, unlike haemolytic disease of the Newborn (Kjaer et al., 2018).

1.5.1.2 Non – immune thrombocytopenia

Non – immune thrombocytopenia is due to increased destruction of platelets. Platelet destruction occurs because of abnormal platelet aggregation and endothelial injury. As a result, fibrin is formed in the arterioles and capillaries which activates platelet consumption. Some of the diseases and conditions reported to be associated with platelet destruction include; disseminated intravascular coagulation, sepsis, thrombotic microangiopathies such as thrombotic thrombocytopenia purpura (TTP) and haemolytic uraemic syndrome (HUS) (Hata, Kimura, Ishii, Suzuki, & Egawa, 2016; Vyas, 2017).

Non – immune thrombocytopenia comprises most of the thrombocytopenic cases admitted in the neonatal intensive care unit (Sola-Visner, 2012; Sola-Visner, Saxonhouse, & Brown, 2008) and presents after 1 to 3 days. Studies have shown that they comprise of more than 80% of the cases (Kaplan, 2008; Roberts et al., 2008) and are assumed to be commonly associated with secondary diseases such as hypoxia, preeclampsia, IUGR, and diabetes (Lambert, 2017; Nguyen, Dugrillon, Beck, Kerowgan, & Kluter, 2004). Other studies have demonstrated that the mechanism of non – immune mediated neonatal thrombocytopenia generally leads to decreased megakaryopoiesis, with cases that tend to be relatively mild and self-limiting (Constantinescu, Zamfirescu, & Vladareanu, 2012; Zdravic et al., 2016).

1.6 Summary

Platelets play a crucial role in haemostasis and are derived from the fragmentation of cytoplasm of megakaryocytes in the bone marrow. The platelet membrane expresses glycoproteins, human platelet antigens (HPA), HLA antigens and ABO antigens. The causes of decrease levels of platelets can be acquired or congenital. Thrombocytopenia is defined as decreased levels of platelets below the reference range of 150 x 10^9/L. It can be mild, moderate and severe. Thrombocytopenia can be classified according to time of onset, namely; foetal, early and late; and immune (auto – or alloimmune) and non-immune.

Chapter 2 Neonatal Alloimmune Thrombocytopenia

2.1 Definition

NAIT is a condition with an estimated incidence in Caucasian women of one in 1000 live births (Crighton et al., 2017; Risson et al., 2012; Sachs & Santoso, 2018). Synonyms for this condition include foetal/neonatal alloimmune thrombocytopenia (FAIT/NAIT) or foeto – maternal alloimmunisation thrombocytopenia (FMAIT) (Kaplan, 2006). It is a potentially life threatening bleeding disorder of the foetus or new born infant (Lin, Xu, Lee, Liang, & Santoso, 2018), and the commonest cause of severe thrombocytopenia (less than 50 x 10^9/L) in healthy newborn babies (Strong & Eddleman, 2013; X. R. Xu, Gallant, & Ni, 2016).

The disease is caused by maternal alloantibodies, specifically immunoglobulin G (IgG) (Sainio et al., 2017), which cross the placenta and target foetal platelets expressing antigens inherited from the father (Refsum et al., 2017) but absent in the mother (Conti et al., 2014). The commonest occurrence is in a mother who is HPA – 1a negative carrying a HPA – 1 a positive foetus. She becomes immunised against the HPA – 1a on the foetal platelets which results in maternal IgG alloantibodies crossing the placenta (Sonneveld et al., 2016), and causing destruction of foetal platelets resulting in thrombocytopenia (Curtis, 2015; Dubruc et al., 2016). This condition may serious bleeding either *in utero* or in the early postnatal period (Vadasz et al., 2015), with potentially severe complications and long – term disabilities (Espinoza, Caradeux, Norwitz, & Illanes, 2013). In addition, it is reported that these alloantibodies not only cause destruction and decrease the production of foetal platelets, but also appear to affect vascular integrity and angiogenesis. This subsequently increases the risk of intracranial and extracranial bleeding complications in foetuses and neonates and may cause imminent intrauterine and perinatal death (Winkelhorst et al., 2016).

Angiogenesis is a biological process whereby new blood vessels are produced from the pre – existing vasculature during foetal development, wound healing and in the formation of granulation tissue. This is a normal physiological and critical process in growth which begins in utero and continues throughout life in both health and disease (Marin-Luevano et al., 2018). This mechanism is regulated by certain angiogenetic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth

factor (bFGF) and epidermal growth factor (EGF) (Duzyj et al., 2018; Zimna et al., 2018).

When oxygen levels in the body are low, sensing mechanisms are activated and result in the secretion of pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF) (Halder, Kant, & Milner, 2018; Zimna et al., 2018). An ensuing cascade of promoters results in the growth of new blood vessels where there were previously none. Once sufficient new blood vessels have been generated, tissue oxygenation becomes sufficient for pro-angiogenic growth factor production to cease and the levels return to normal (Liakouli et al., 2018).

NAIT is a relative rare disorder but a leading cause of morbidity and mortality in neonates. The most devastating effect of NAIT is intracranial haemorrhage (ICH) in term infants (Espinoza et al., 2013; Hutchinson et al., 2015). The majority of reported cases are Caucasian (Portela et al., 2016), although clinically significant ethnic variations in platelet genotypes occur and may result in NAIT (Risson et al., 2012).

Although the disease is potentially dangerous, it is still under-diagnosed (Conti et al., 2014). In the majority of cases, NAIT is considered mild (Delbos et al., 2016) and remits spontaneously; however, thrombocytopenia can be severe and fatal (Bakchoul et al., 2014). Studies have demonstrated that cases with severe neonatal thrombocytopenia (Penel-Page et al., 2017), tend to resolve gradually postnatally as maternal antibodies, usually IgG, are cleared from the system. This may take up to two or three months or longer (Sillers et al., 2015).

At present, there is no national screening program for NAIT in New Zealand. The diagnosis is suspected from the clinical presentation and sometimes a history of previous affected pregnancies.

2.2 Pathogenesis

2.2.1 Clinical Features

Clinical features and symptoms of NAIT vary from a mild petechial rash, bruising or purpura to more serious incidents of bleeding in the gastrointestinal tract, kidneys and brain where fatal intracranial haemorrhage (ICH) may occur (Barg et al., 2017; Curtis,

2015; Dubruc et al., 2016). Foetal bleeding utero causes miscarriages and preterm births (Charoenkwan et al., 2015; Nakamura et al., 2015).

The frequency of miscarriages in mothers of infants with NAIT has not been reported but they are known to occur. It is likely that the incidence is under – reported, and the collective rate of miscarriage in affected pregnancies has not been addressed (Silver et al., 2007; Zdravic et al., 2016).

NAIT is the leading cause of ICH in term infants (Hutchinson et al., 2015). Intracranial haemorrhage continues to be the most severe clinical complication of NAIT in newborn infants. Although the incidence of ICH in newborn infants with this condition is low, it can often be fatal (Tiller, Husebekk, Skogen, Kjeldsen-Kragh, & Kjaer, 2016). Haemorrhaging can happen as early as the 14th week of pregnancy *in utero*. It is therefore essential to establish early and proper diagnosis and management in these infants. This is to prevent significant offspring morbidity and mortality (Bakchoul et al., 2013; Salomon & Rosenberg, 2013; Zdravic et al., 2016).

Foetal platelet antigens are expressed as early as 16 weeks of gestation by which time may have entered the maternal circulation. Moreover, pre-existing maternal anti – HPA antibodies can persist for many years. Further exposure to corresponding antigen on foetal cells in subsequent pregnancies can stimulate an enhanced immune response (Silva et al., 2011). Studies have demonstrated that infants who develop NAIT in subsequent pregnancies tend to have a similar or even more severe degree of thrombocytopenia than earlier siblings (McQuilten, Wood, Savoia, & Cole, 2011). The most significant clinical sign for the severity of the disease in subsequent pregnancies is ICH in previous siblings (Tiller et al., 2016). Furthermore, the presence of ICH in some cases is independent based on the degree of thrombocytopenia in the offspring which may be due to other pathological conditions other than bleeding due to abnormally low platelet counts (Hopkins et al., 2017).

On the other hand, anti – HPA – 1a antibodies mediate phagocytosis of platelet by monocytes while supressing foetal megakaryopoiesis (Pluthero & Kahr, 2018). Meanwhile studies have shown that certain subtypes of anti-HPA-1a antibodies are associated with the presence of ICH thus reducing the proliferation of endothelial cells and angiogenesis (Refsum, Meinke, et al., 2018).

2.2.2 HPA subtypes

In NAIT maternal anti – HPA antibodies target the foetal antigens (HPA) that are inherited from the father (Refsum, Meinke, et al., 2018). This most commonly occurs when a mother who is HPA – 1a negative carries a HPA – 1 a positive foetus and is immunised against the HPA – 1a on foetal platelets resulting in maternal IgG alloantibodies crossing the placenta (Conti et al., 2014; Refsum et al., 2017; Sainio et al., 2017; Sonneveld et al., 2016). The specificity of maternal alloantibody corresponds to an HPA designation in which a high frequency allele is termed 'a' and a low frequency form 'b'. To date, antibodies to more than thirty-five (35) of the known HPAs have been reported as causative agents of NAIT since it was first described in the 1960s (Curtis, 2015). Antibodies against HPAs are designated anti – HPA.

Most literatures reported that anti – HPA antibodies are identified as causes of NAIT (Espinoza et al., 2013). In addition, they are reported to cause other clinical conditions such as post transfusion purpura, and platelet transfusion refractoriness (Londero et al., 2018; Matsuhashi et al., 2014). Human platelet antigen differs in different ethnic population therefore causes differences in the incidence of anti – HPA antibodies and risk of NAIT (Chen et al., 2017).

The most common cause of NAIT in approximately 80% of the Caucasian population is antibodies to HPA – 1a followed by HPA – 5b, whereas in Japanese and other Asian populations, it is anti - HPA – 4b and HPA – 6b antibodies (Edinur, Dunn, Lea, & Chambers, 2013; Lin et al., 2018). Antibodies to other HPAs such as HPA – 5b, - 1, - 2, - 3, and - 15 have been implicated in Asian, African, Middle Eastern, Pacific, American and European populations (Al-Ouda et al., 2016; Tiller et al., 2012; X. Xu et al., 2018). In particular, and relevant to New Zealand clinicians, a higher frequency of anti - HPA – 15 antibodies is reported to be present in Polynesian and Maori people (Edinur et al., 2013). However, it remains controversial whether the antigens implicated are similar to the Caucasian population (Hutchinson et al., 2015), since Maori and Polynesian mothers do not appear to have the same risk factors. Few studies have been conducted on the frequency of the alleles for HPA – 15 (Edinur et al., 2013), however, antibodies to these low frequency HPA antigens are also capable of causing NAIT (Peterson et al., 2014).

Since New Zealand is a multicultural country and intermarriage is common, knowledge of the relative prevalence of different HPA amongst ethnic groups would be clinically useful. Given the different population groups namely; Polynesians, Maori, Asians, Africans and Europeans with differing HPA alleles prevalence, the risk of platelet specific alloimmunization is high (Edinur et al., 2013; www.adhb.govt.nz, n.d).

2.2.3 Mechanism of antibody development in NAIT

NAIT occurs when foetal platelet alloantigen's inherited from the father (Zdravic et al., 2016) and absent in the mother (Conti, 2013) stimulate a maternal immune response against those foetal platelet antigens resulting in foetal thrombocytopenia (Espinoza et al., 2013). Although it has clearly been demonstrated to be caused by incompatibility of maternal and foetal alloantigens, the mechanism of exposure is not fully understood (Delbos et al., 2016).

The pathophysiology of NAIT is analogous to that of haemolytic disease of the new born (HDN), except that NAIT affects platelets whereas HDN affects red cells (Brojer et al., 2016). Unlike HDN, NAIT affects the first pregnancy, and tends to cause more severe thrombocytopenia in subsequent pregnancies (McQuilten et al., 2011). Immunisation against HPA in NAIT can be a result of foetal - maternal haemorrhage during birth (as with HDN), however, the mechanism is unclear (Refsum, Meinke, et al., 2018; Zdravic et al., 2016). On the other hand, Curtis (2015) reported that maternal sensitisation occurs as a result of interaction with foetal cells during foetal maternal haemorrhage or delivery. Maternal sensitisation is also reported to occur during the shedding of trophoblast microparticles into the maternal circulation. It is however reported that not all mothers exposed to incompatible HPA produce antibodies. This occurs in HPA – 1a negative women and is only exposed during pregnancy if the father is heterozygous or homozygous. In fewer cases they are incompatible due to other HPAs.

Since NAIT usually occurs in the first pregnancy, early release of foetal platelets into the maternal circulation may occur, thus triggering an immune response. The high recurrence rate, often with progressively more severe manifestations in subsequent pregnancies, is thought to be a result of enhanced secondary immune response (Brojer et al., 2016; Espinoza et al., 2013). Immunisation against platelet antigens in NAIT may

be attributed to the smaller size and thus greater mobility, of platelets. Studies have reported that human placental trophoblast cells (Eksteen et al., 2017) express β3 integrin, prompting the suggestion that an immune response may be generated independently from platelets (Espinoza et al., 2013; Vadasz et al., 2015). Furthermore, several studies have demonstrated that spermatozoa express β3 integrin suggesting that woman may even become immunised to foreign antigen during preconceptional exposure (Espinoza et al., 2013). Whenever exposure to maternal circulation occurs, platelet foreign antigens are processed by the antigen presenting cells in lymphoid tissue and these then present the antigens to T helper lymphocytes (Tsuno et al., 2014). The T cells then stimulate and enhance B cells to become activated and differentiate into plasma cells, which secrete antigen-specific IgG antibodies (Lin et al., 2018).

Studies have shown that about one third of the women who are HPA – 1 negative (HPA – 1bb) are positive for HLA – DR antigen B3*0101. When exposed to the incompatible HLA antigens it poses a high risk or danger that a woman will become immunised during pregnancy against HPA – 1a when she carries an HPA – 1a positive foetus (Espinoza et al., 2013; Peterson et al., 2013; Zdravic et al., 2016). Although HLA antibodies are frequent in multiparous women, differences have been implicated in NAIT, although the role of anti – HLA antibodies in this disorder is controversial (Meler et al., 2017). The latter study demonstrated that cord blood analyses do not demonstrate all HLA antibodies and only a few cases of NAIT have occurred in women who are positive for HLA antibodies. They further suggested that a possible explanation for this scenario is that anti – HLA antibodies are absorbed by HLA antigens on placental tissue. These therefore are unable to cross into the foetal circulation in inadequate numbers to abruptly reduce the number of platelets (Meler et al., 2017).

When antibodies against foetal alloantigens are produced in the mother and enter the foetal circulation, foetal platelets are opsonised and removed by the reticuloendothelial system (McQuilten et al., 2011). Platelet destruction in NAIT has been demonstrated to be similar to that in ITP. In this condition, thrombocytopenia is usually due to autoantibody production, but alloantibodies can also be produced by mothers with ITP (Brojer et al., 2016).

The Fc receptors on macrophages bind to the Fc fragment of IgG bound to platelets which are then cleared by the reticuloendothelial system in the spleen (Zdravic et al., 2016). However, some patients with ITP have been shown to have antibodies targeting

GPIbα but with Fc – independent platelet clearance. When the maternal platelets that are coated with IgG autoantibodies enter foetal circulation, they bind to Fcγ receptors on the syncytiotrophoblast cells of the placenta. These trigger the foetal antigens to cross react thus causes foetal thrombocytopenia. These platelets are then cleared through Fcγ receptor – mediated phagocytosis by macrophages and usually occurs in the spleen and liver which then triggers the maternal circulation to rapidly clear the platelets (Kohli & Isermann, 2017; Perepu & Rosenstein, 2013; Pluthero & Kahr, 2018).

It is thought that this mechanism may also occur in NAIT. Thus, controversy remains regarding significant differences between the means of adult and foetal/neonatal platelet clearance (Curtis, 2015).

2.2.4 HLA

HLA antigens are expressed on all nucleated cells and platelets, however, exposure to the incompatible HLA antigens triggers the host immune system that in turn produces alloantibodies (Dahl et al., 2016). Although the HLA Class I antigens are expressed on the platelet membrane, their role in NAIT is controversial (Hutchinson et al., 2015). It is reported that maternal anti-HLA Class I antibodies can cause destruction of platelets and hence causes NAIT in the foetus the mother is carrying (Kjaer et al., 2018).

On the other hand, Refsum et al. (2017) documented that HLA Class I antigens can provoke foetal thrombocytopenia in some cases. However, this was critiqued by Abraham, Chacko, Fouzia, Srivastava, and Daniel (2018) that anti – HLA antibodies are often associated with platelet alloimmunisation and also involved in producing antibodies against other diseases such as platelet refractoriness.

Maternal HLA Class I antigens are usually detected in multiparous women. Most of the causes of clinically significant NAIT are attributed to HLA (Peterson et al., 2013). Although, anti – HLA significantly pose harm in certain diseases such as platelet refractoriness following transfusion and rejection of allografts, there is controversy on their role during pregnancy (Dahl et al., 2016; Dahl et al., 2017).

2.2.5 ABO antigens

Studies have reported that there is a correlation between maternal ABO phenotype and genotype and severity of thrombocytopenia of newborn infants (Ahlen et al., 2012). In addition, women with blood type O have a lower risk of having a child with severe NAIT than women with blood group A. The severity of NAIT due to anti – HPA – 1a antibodies is observed to correlate with maternal ABO blood types, though it is argued that this is due to genetic properties on the maternal side. However, the occurrence of NAIT due to ABO incompatibility is rare (Bussel & Primiani, 2008).

2.3 Diagnosis and Management of NAIT

2.3.1 Investigation of NAIT

The diagnosis of NAIT is often first considered when there is an incidental finding of low platelet count in an otherwise healthy infant, or when a symptomatic newborn infant presents with petechial rash and purpura. More serious presentations include intracranial haemorrhage or other serious bleeding at or within a few hours of birth (Kaplan, 2008; Orzechowski, 2016). In addition, where there is a previous family history of NAIT, this diagnosis should also be considered (Mella & Eddleman, 2015).

The differential diagnosis includes other causes of neonatal thrombocytopenia such as sepsis, DIC, and other congenital conditions associated with thrombocytopenia (G. Bertrand, Kaplan, C, 2014; Gunnink et al., 2014).

The diagnosis of NAIT is based on clinical and serological findings and includes demonstration of thrombocytopenia in new born infant, HPA genotyping, HPA antibody screening of the mother, and paternal platelet cross match (Silva et al., 2011; X. Xu et al., 2018).

Although there is no screening program for NAIT in New Zealand, the New Zealand Blood Service (NZBS) performs the following tests for NAIT investigations: HPA genotyping, HPA antibody screening of the mother and paternal platelet cross-match.

HPA genotyping is used for typing the mother, father and child. It is important that the child is typed because if this is not performed, then HPA mismatches may only be

inferred (but not proven) (Pai, Burnouf, Chen, & Lin, 2013). The method used is the Linkage Biosciences LinkSēq™ HPA genotyping kit using real time polymerase chain reaction (RT – PCR). This is conducted in the Tissue Typing Laboratory at NZBS to determine the human platelet antigen (HPA) genotypes of platelet and plasma apheresis donors and for the investigation of neonatal alloimmune thrombocytopenia and platelet transfusion refractoriness (Reiher et al., 2017).

HPA antibody screening of the mother is undertaken to ascertain if she has any HPA antibodies against the child, or against any HPA mismatches she may have against the partner which could have been inherited by the child (van den Akker & Oepkes, 2008).

In addition, a cross match test is performed using maternal serum and paternal platelets. This method is performed to identify any reactivity between the serum of the mother and the partner's platelets (Hopkins et al., 2017). This is a non-specific test which if positive could be due to HPA antibodies, but also HLA antibodies or ABO incompatibility. It can help, however, in conjunction with the other test results, to indicate the type of mismatch/ antibodies present (Del Vecchio, Motta, & Romagnoli, 2015).

2.3.2 Management of NAIT.

Currently there are no clear evidence-based guidelines for managing NAIT, and most institutions adopt their own threshold platelet counts below which decisions are made for the administration of platelet transfusions and/or high dose intravenous immunoglobulin (IVIG) (Risson et al., 2012).

Since most countries do not have a screening program, many cases of NAIT are detected incidentally and treated or according to the severity of bleeding complications, which range from bruising or petechiae to intracranial haemorrhage in the foetus. Women who have had a previous case of an affected infant are ideally identified and monitored (Winkelhorst et al., 2017).

The goal of management plans is to offer pregnant women carrying an infant at risk of developing NAIT, treatment, aimed at preventing the development of severe thrombocytopenia in an affected infant, who might be at risk of having ICH and its

sequelae, including death. These complications can occur either antenatally or after birth (Brouk, 2015).

A variety of treatment options exist, the choice partly dependent on the severity of NAIT in previously affected siblings. With advancing technological interventions, there has been a continuing change in antenatal treatment ranging from invasive management protocols to less invasive and completely non-invasive approach (Winkelhorst et al., 2016).

Controversy remains over the optimal antenatal management strategy. It is essential that laboratory results are evaluated, and if the mother and foetus are incompatible, ongoing treatment and surveillance is required (Tiller et al., 2016).

Several antenatal treatments have been shown to improve the platelet count in a foetus at risk of NAIT and are useful in managing the disease and subsequent pregnancies (Abraham et al., 2018; Londero et al., 2018).

Intravenous immunoglobulin (IVIG) and corticosteroids

For more serious cases, the administration of IVIG in high doses is recommended. A demonstrable improvement in foetal platelet count can be expected with this type of treatment (Skariah et al., 2017).

IVIG is the primary treatment option for decreasing the destruction of foetal platelets by blocking the immune response to maternal antibodies. However, its exact mechanism of action remains controversial but includes a range of immumodulatory actions operating alone or in combination (Rossi, Lehman, & O'Shaughnessy, 2016).

Since most countries do not have a screening program, there are no guidelines for the optimal dose of IVIG nor the ideal time frame for treatment to commence according to gestational age (G. Bertrand, Drame, Martageix, & Kaplan, 2011).

Corticosteroids

Corticosteroids have been reported to improve foetal platelet counts but the ideal dose remains uncertain in terms of balancing benefits against risks of such treatment (G. Bertrand, Kaplan, C, 2014).

Platelet transfusions

Kaplan (2008) recommends platelet transfusions for infants with clinical bleeding or severe thrombocytopenia during the first 24 hours of life. These platelets should be antigen – negative from phenotyped donors. Other authors, such as Sharma and Thapar (2015), support this idea and in fact state that the only suitable treatment for neonatal thrombocytopenia is platelet transfusion. Treatment is administered to reduce the risk of severe bleeding (Kaushansky, 2008), and correct platelet function defects (Wang, Yang, Stevens, & Wang, 2017). Platelet transfusions containing healthy allogeneic platelets can partially alleviate and correct any platelet functional defects however, there are no studies on the association of the platelet function defect and immune thrombocytopenia (Fiore, d'Oiron, Pillois, & Alessi, 2018). Although, platelet transfusion is considered to reduce the risk of bleeding, it is less ideal as platelet associated antigens vary among different races and therefore transfusion of compatible platelets can be difficult (Wang et al., 2017).

Whatever treatment options are applied, the most important thing in all cases is to monitor the neonate closely until the platelet count reaches a safe level (Kaplan, 2008).

In addition, referral of the mother for antenatal monitoring of future pregnancies is strongly recommended.

Chapter 3 Rationale, Hypothesis, and Research Objectives

3.1 Rationale

There is no screening program for NAIT in New Zealand and therefore the incidence is unknown. Currently the diagnosis is mostly assumed, based on exclusion of other known causes of thrombocytopenia. Thus, the disease is not properly diagnosed. Moreover, there is often no follow-up of the mother for subsequent pregnancies during which the foetus might be more severely affected.

Local knowledge of the incidence and outcomes of thrombocytopenia in healthy term infants in New Zealand is crucial to improving rates of detection, diagnosis and subsequent management of NAIT. The identification of possible risk factors for affected infants would also be useful in developing protocols for screening and management. As NAIT is an uncommon disease, a large number of cases must be analysed in order to provide meaningful data.

3.2 Research Question

NAIT is rare and remits spontaneously. It is the commonest cause of thrombocytopenia in a healthy term infant. What is the prevalence of neonatal thrombocytopenia at birth in infants born at Middlemore Hospital, and how many cases are likely to be due to NAIT?

3.3 Research Aim & Objectives

3.3.1 Aim

To determine the prevalence of NAIT in cord blood of infants born in Middlemore Hospital, and correlate with their clinical outcomes, findings of which will be made known to appropriate authorities so that appropriate action can be taken.

3.3.2 Objectives

A retrospective study is designed to determine the prevalence and clinical outcomes of thrombocytopenia in healthy term infants born at Middlemore Hospital.

Specifically, it aims to:

3.3.2.1 Determine the prevalence of thrombocytopenia in otherwise healthy term infants of varying ethnicities.

3.3.2.2 Investigate associations of thrombocytopenic infants with clinical outcomes in affected infants and subsequent siblings.

3.3.2.3 Develop a suggested guideline for the future detection and management of NAIT in New Zealand.

3.3.2.4 Identify cases of NAIT in asymptomatic infants

Chapter 4 Methodology

4.1 Ethical Clearance

Ethical clearance for this research was approved by the Research Committee of Middlemore Hospital, Auckland, New Zealand, under the condition that an employee working in that hospital acts as supervisor to the principal investigator and be the medium of communication with the committee. The notification indicated that in order to use the hospital premises the following processes be completed: provision of access and ID card; provision of a clearance for a Police Vetting and Vulnerable Children's Act (VCA); completion of an Application for Volunteers and provision of a login password to access the Concerto data base.

4.2 Police vetting and Vulnerable Children's Act

Police vetting aims to safeguard the community and its most vulnerable people. These include children, older people and people with special needs. This process allows organisations to make appropriate decisions regarding potential employees or volunteers who work directly with vulnerable people.

The audit involved children; therefore, it was required to fulfil requirements under the VCA. The Faculty of Health and Environmental Sciences (FHES), Auckland University of Technology (AUT), requires that if the research planned by enrolled students involves children they are required to undertake police vetting and VCA. The faculty requirements to address the VCA are proof of identity, police vetting in compliance with the VCA, reference checks and curriculum vitae (CV) including the previous 5 years. The process involves completion of an addendum, which requires consent. This was completed through the Student Hub of Auckland University of Technology.

An appropriate letter was provided. This stated that the student doing the research had undergone a full safety check including NZ Police Vetting under the VCA 2014, interviewing, reference checking, and ID verification. These checks identified no concerns and were accepted by the Research Committee at Middlemore Hospital.

4.3 Application for volunteers

Permission to work as a volunteer at Middlemore Hospital involves the collection of information about the person doing the research. This is being used by Counties Manukau District Health Board (CMDHB) on an ongoing basis for the purpose of assessing the characters of volunteers. The information is available to the manager and is kept confidential and secure.

4.4 ID and Access card

After completion of the relevant paper work, permission was granted to use the hospital premises, particularly the Haematology laboratory section, and the investigator was issued with ID and access card.

4.5 Sampling

4.5.1 Retrospective data collection.

Relevant information was gathered from the Concerto database and access to the files was provided by personnel in the Haematology laboratory. After collection, all patient identities were removed from the report.

The data set include the full blood count (FBC) results of cord blood samples from all babies born at Middlemore Hospital between January 2005 and mid – 2016.

Clotted and clumped samples did not contain conclusive results for platelet counts for the infants therefore this could have reduced the total number of thrombocytopenia cases or cause spurious results for other haematology parameters. In addition, they were rejected for analysis and requested for a repeat sample.

All babies with a platelet count below the reference range (150×10^9/L) were selected. In addition to FBC results, relevant clinical data was obtained from the clinical records of the selected babies and their mothers, available through the hospital Concerto database. Data collection for the audit was conducted following an algorithm for retrospective analysis as shown in Figure 4.1.

Those thrombocytopenic infants that were not regarded as healthy term infants had their platelet count recorded and documented. This was to determine if the infants were asymptomatic or symptomatic. The infants were recorded as asymptomatic if their report did not show evidences of other medical conditions other than thrombocytopenia. The infants that ~~have~~ reported evidences of medical conditions and thrombocytopenia were recorded as symptomatic infants. For the symptomatic infants the information gathered included reasons for admissions, primary diagnosis and secondary diagnosis.

The information gathered includes: number and type of any bleeding events, specific treatment prescribed, subsequent platelet counts, number of days in hospital, postnatal follow-up, ethnicity, parity of the mother, any illness during pregnancy, and whether subsequent babies were born to the same mother (in which case these cases were also included in the audit).

Cases with thrombocytopenia attributable to other known causes (e.g. asphyxia, sepsis, necrotising enterocolitis, maternal immune thrombocytopenic purpura (ITP), drugs and rare disorders) were documented but excluded from analysis.

The main focus of this audit was to identify NAIT cases in asymptomatic and symptomatic thrombocytopenic infants.

Where the information was not recorded in the Concerto database, another database was used to find it. The Healthware database was used for obtaining the National Health Index (NHI) for the infant's family.

Using the NHI of the thrombocytopenic infants, the Healthware database was used for recording the NHI numbers of the mother and the siblings of known NAIT cases and asymptomatic thrombocytopenic infants. Since "non-NAIT" causes of thrombocytopenia in symptomatic infants are relatively common, this group was not selected to explore maternal and sibling data. After gaining access to Healthware database, the infants information's were gathered including gestation age for those infants recorded as unknown gestation age, NHI numbers and ethnicities for the mother for asymptomatic thrombocytopenic infants, NHI numbers for the asymptomatic thrombocytopenic infant's siblings. Once their NHIs were gathered Concerto database was used to find information on the clinical details and thrombocytopenia.

Information collected included whether the mother had any family history of NAIT and any diseases known to be associated with thrombocytopenia. Further data included the presence of any thrombocytopenic siblings, a record of ICH and any serological confirmation of NAIT.

Asymptomatic infants were identified to determine if they had thrombocytopenia, and whether the mother had a prior record of any still births, miscarriages or other medical conditions. For asymptomatic infants, the ethnicities of the mothers were documented.

Most thrombocytopenic infants did not have a record of gestational age in the Concerto database therefore Healthware was used to find this information.

NHI numbers obtained in the Healthware and Concerto databases were used to document platelet count, clinical details and diseases associated with thrombocytopenia in the mothers and siblings.

Each thrombocytopenic infant was assigned numbers according to severity starting with number 1 up to 794. The numeral number 1 indicates that the condition is severe, while 794 indicates a mild thrombocytopenia.

4.6 Observation of diagnostic tests for NAIT at New Zealand Blood Service

A visit to New Zealand Blood Service (NZBS) was arranged by a charge scientist of the Tissue Typing laboratory to observe the diagnostic methods for NAIT. The aim of this visit was to observe which methods are performed for NAIT and for how long the laboratory has been conducting these tests. During the visit, a brief orientation of the laboratory was conducted, and the methods were explained in detail, covering from how they are performed and for what conditions.

The scientist explained that the following methods are performed at the laboratory;

4.6.1 IMMUCOR PAK - LM™ (Luminex Assay) test for the detection of serum platelet antibody

This method is performed to detect serum platelet antibodies. It pertains to test requests when platelet antibody screening is required for investigations of allo or auto-immune thrombocytopenia by the National Tissue typing Laboratory at NZBS.

4.6.2 Linkage Biosciences LinkSēq™ HPA genotyping kit.

This method is performed to determine the HPA genotypes of platelet and plasma apheresis donors and for the investigation of neonatal alloimmune thrombocytopenia and platelet transfusion refractoriness where required.

4.6.3 Flow cytometry platelet immunofluoresence test.

Flow cytometry platelet immunofluoresence test (FC – PIFT) is used to test for anti – platelet antibody and the test is used to test for serum platelet antibodies (SPA) and platelet associated antibodies. The procedure for testing SPA is specifically investigating for HPA antibodies including dependent serum platelet antibodies whereas testing for platelet associated aims to determine if any immunoglobulin is attached to the patient's own platelets. The procedures for testing platelet associated antibodies include paternal platelet crossmatch and HPA – 1a genotyping and phenotyping. Paternal platelet crossmatch is performed on the maternal serum against paternal platelets and is performed to see if there are direct incompatibility between parents that may indicate feto – maternal platelet incompatibility. On the other, HPA – 1a phenotyping is a serological technique for phenotyping maternal and paternal platelets. This is important in NAIT investigations.

The sensitivity of FC - PIFT is about 85% and specificity 75%, whereas the sensitivity of PAK – LXTM is around 98% sensitivity and close to 100% specificity. The specificity refers to an ability of a diagnostic test in accurately identify true positive and negative values whereas sensitivity is the probability that a diagnostic test accurately identify the true positive value (Bub et al., 2013).

4.7 Data Analysis

Biostatician was consulted to assist in the statistical analysis of the data and to recommend which statistical methods were relevant for the data. The biostatistician recommended that relative risk calculation and 95% confidence interval (CI) should be used to compare differences in gestation age and gender and to determine which gender is more likely to develop thrombocytopenia.

Microsoft Excel was used for categorising most of the data such as total thrombocytopenia, total severity of thrombocytopenia, gestation age, symptomatic and asymptomatic thrombocytopenic infants, follow up of repeat platelet count, and clinical admissions.

Relative risk (RR) was used in this study to compare associations of thrombocytopenia with gender and gestational age groups (preterm and term). A 95% Confidence Interval (CI) was used in conjunction with RR to determine where the overall thrombocytopenia cases would lie. The values were calculated using online (gigacalculator.com, 2018) calculators and since this is the most frequent report CI level.

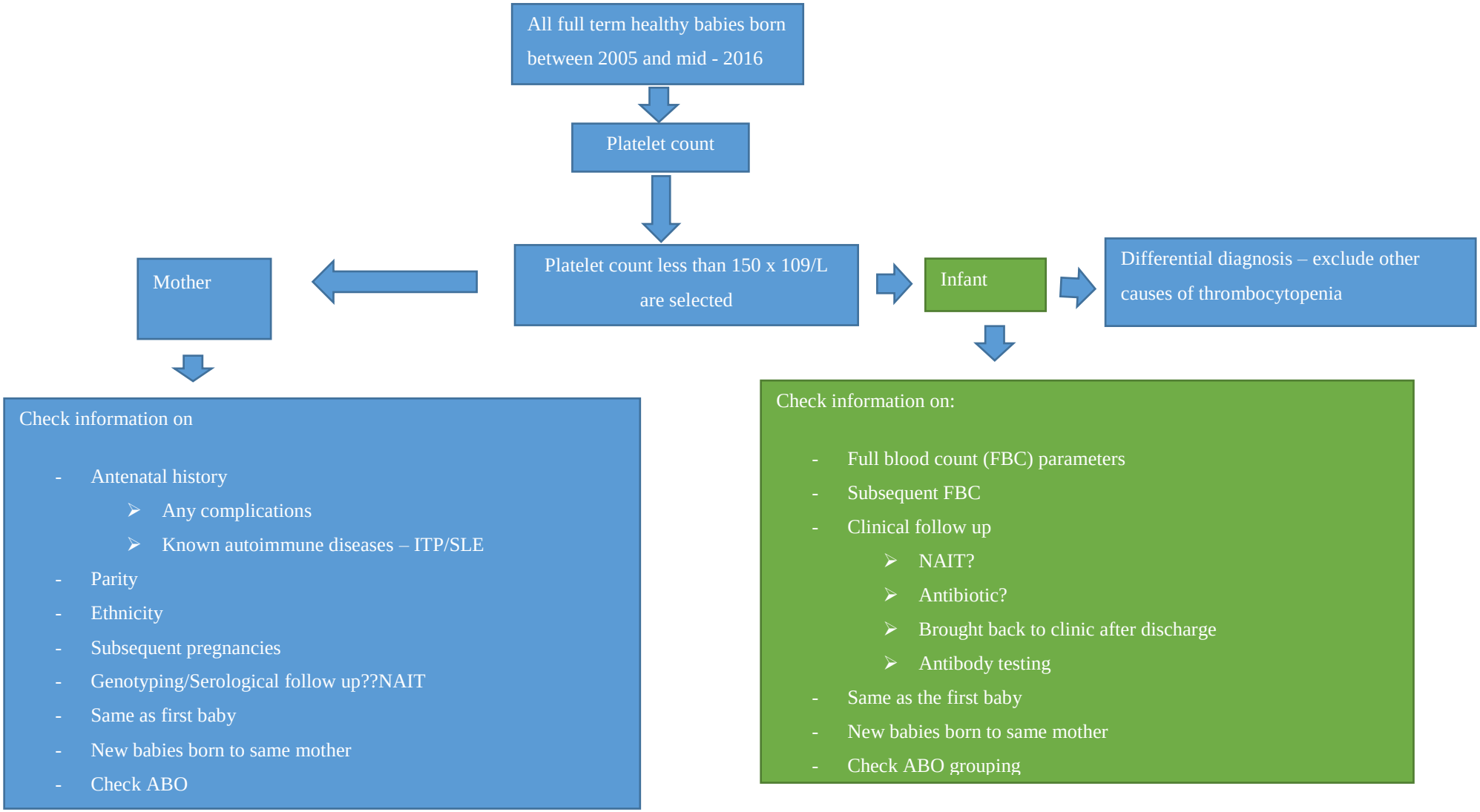

Figure 4.1. Algorithm of data for retrospective analysis. FBC: full blood count, NAIT: neonatal alloimmune thrombocytopenia, SLE: Systemic Lupus Erythematosis, ITP: Idiopathic Thrombocytopenic Purpura.

Chapter 5 Results

5.1 Total number of births

A total of 68910 births were recorded at Middlemore Hospital between 1st January 2005 and 30th June 2016.

5.2 Cord full blood counts from 2005 to June 2016.

During this time, 66109 cord blood full blood count (FBC) requests were processed and recorded at Middlemore Hospital Haematology laboratory. Of these requests, 89.9% had a normal platelet count, 1.2% were thrombocytopenic, 2.8% revealed a thrombocytosis, and 6.1% had no conclusive results due to clumped and clotted samples (Table 5.1 and Figure 5.1).

Table 5.1. Total FBC request from 2005 to June 2016 (n = 66109)

Normal	Thrombocytopenia	Thrombocytosis	Clumped	Clotted or other reasons
59422	799	1862	387	3639
89.9%	1.2%	2.8%	0.6%	5.5%

5.3 Thrombocytopenic Infants

5.3.1 Severity, Gender and gestational age

The 799 thrombocytopenic samples were categorised as severe if the platelet count was less than 50 x 10^9/L (<50 x 10^9/L), moderate if the platelet count was equal to or greater than 50 but less than 100 x 10^9/L (≥50 to <100 x 10^9/L) and mild if the platelet count was equal to or greater than 100 but less than 150 x 10^9/L (≥100 to <150 x 10^9/L). The results are tabulated in Table 5.2.

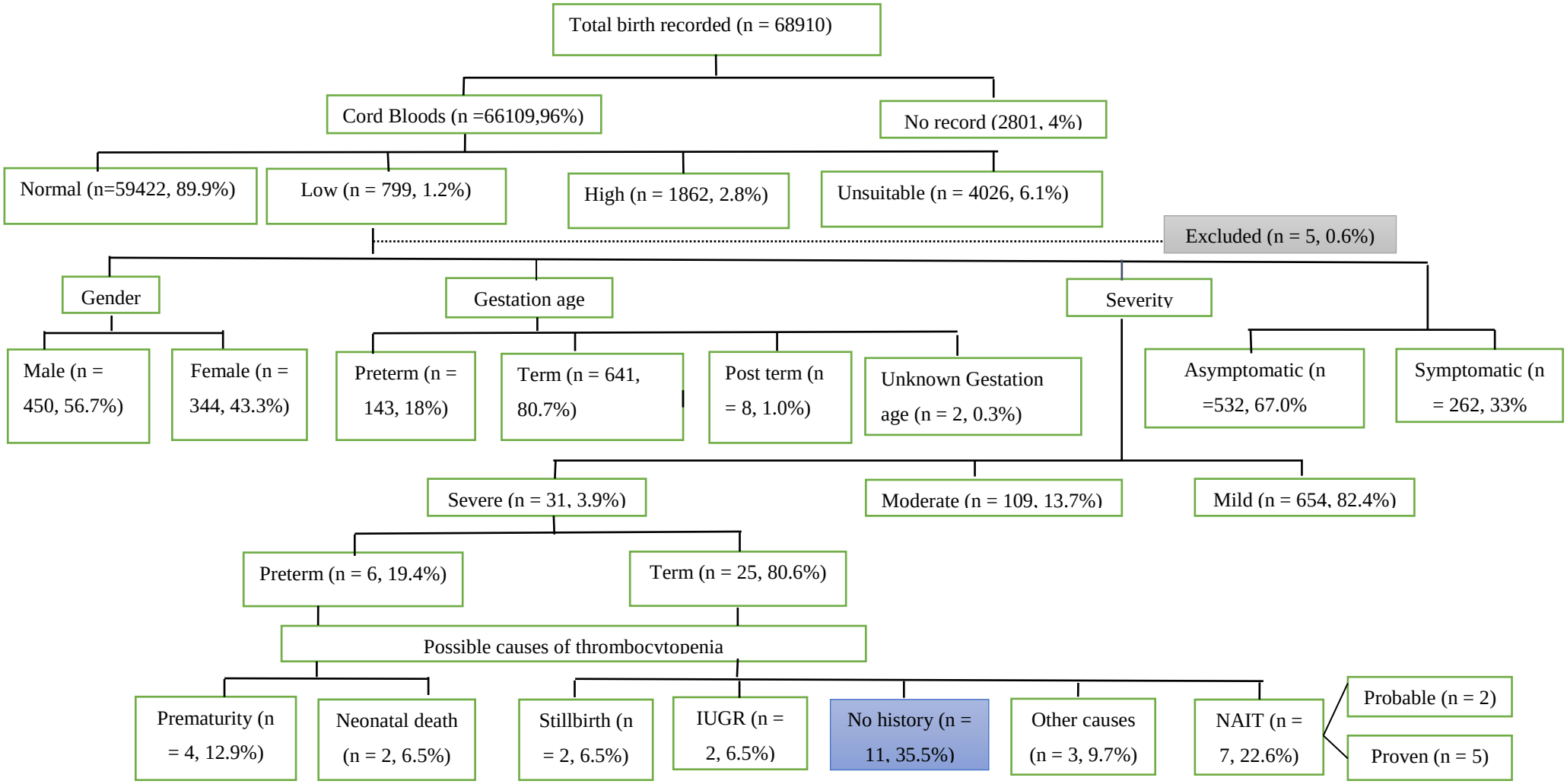

Figure 5.1. Flowchart for Total Cord blood samples and thrombocytopenia

Table 5.2. Thrombocytopenic infants according to severity, gender and gestational age.

	Severity of thrombocytopenia																	
	Severe (n = 31, 3.9%)				Moderate (n = 109, 13.7%)				Mild (n = 654, 82.4%)				Total (n = 794, 1.2%)					
Gestation	**Male**	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**Total**	**%**
Preterm (<37 weeks)	4	0.5	2	0.3	14	1.8	12	1.5	59	7.4	52	6.5	77	9.7	66	8.3	143	18.0
Term (37 - 42 weeks)	15	1.9	10	1.3	43	5.4	39	4.9	310	39.0	224	28.2	368	46.3	273	34.4	641	80.7
Post term (>42 weeks)	0	0.0	0	0.0	1	0.1	0	0.0	4	0.5	3	0.4	5	0.6	3	0.4	8	1.0
Unknown gestation age	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	0	0.0	2	0.3	2	0.3
Total	**19**	2.4	**12**	1.5	**58**	7.3	**51**	6.4	**373**	47.0	**281**	35.4	**450**	56.7	**344**	43.3	**794**	

Five infants were excluded from the total number of thrombocytopenic infants (n = 799). In one case this was because an infant was recorded twice as mild thrombocytopenia using the same NHI but different laboratory request numbers. Thus, the second set of haematology results were excluded. In the remaining four infants there was no record of gender, gestational age or birthweight. Of these four infants, three infants had mild and one had moderate thrombocytopenia (Figure 5.1).

Of the 794 thrombocytopenic infants, 2.4% with severe thrombocytopenia were males and 1.5% were females; 7.3% with moderate thrombocytopenia were males and 6.4% were females; 47.0% with mild thrombocytopenia were males and 35.4% were females (Table 5.2)

All thrombocytopenic cases were further classified as preterm, if the infants were born less than 37 weeks of gestation, term, if the infants were born between 37 and 42 weeks of gestation, and post – term, if the infants were born after 42 weeks of gestation. The infants categorised as unknown gestational age refers to cases where there is no record of gestational age (Table 5.2 and Figure 5.1).

The majority of the thrombocytopenic cases were term infants (80.7%), followed by preterm (18.0%), post term (1.0%), and unknown gestational age (0.3%). Of the 31 severe cases, 80.6% were term infants, and 19.4% were preterm. None of the post term infants nor infants with unknown gestational age had a severe thrombocytopenia (Table 5.2 and Figure 5.1).

5.3.2 Relative risk

Table 5.3 **Relative risk**

	Preterm	**Term**	**Total**
Male	77	373	450
Female	66	278	344
	143	641	794

Calculation of Relative risk (RR)

Relative risk of preterm infants

RR: preterm male/total male ÷ preterm females/total female infants

= (77/450) ÷ (66/344)

= 0.17/0.19

= **0.89**

95% Confidence Interval (CI) (0.662, 1.201).

A relative risk of less than 1, indicates that there is a lower risk of term males developing thrombocytopenia than their preterm counterparts. The CI of the RR of thrombocytopenia in males compared to females is between 0.7 and 1.2.

5.3.3 Term infants

Twenty-five term babies had a severe thrombocytopenia at birth. Based on the recorded primary diagnosis, five cases (numbers 1, 2, 4, 14 and 17) were proven to have NAIT. Another two cases (numbers 18 and 26) were suspected to have NAIT based on the clinical findings. One infant (number 18) was recorded as "probable NAIT with ventricular septal defect" (VSD) and yet another (number 26) appeared clinically to be NAIT, but diagnostic testing failed to confirm this (

Table 5.5 and Figure 5.1).

Eleven infants had no recorded medical history other than severe thrombocytopenia, two were diagnosed with intrauterine growth retardation (IUGR), two were stillbirths, one had bilirubin encephalopathy/kernicterus, one had sepsis, and one was diagnosed with haemolytic disease of the newborn (HDN) (

Table 5.5 and Figure 5.1).

5.3.4 Preterm infants

Of the six severely thrombocytopenic preterm infants, four had a primary diagnosis of prematurity and two were recorded as “early neonatal death” due to antenatal asphyxia and extreme prematurity (Table 5.4 and Figure 5.1).

5.3.5 Moderate and Mild thrombocytopenia

Due to the large numbers of babies with moderate and mild thrombocytopenia, it was decided not to analyse clinical history on these individuals.

Table 5.4. Preterm infants with severe thrombocytopenia at birth.

Table 5.5. Term infants with severe thrombocytopenia at birth.

Infant No	Sex	GA	BW (g)	Reasons for Admission	Platelet Count	Primary Diagnosis	Secondary Diagnosis	Outcome and record of resolution of thrombocytopenia
5	M	36	3150	Not recorded	8	Not recorded	Not recorded	Neonatal death after day 3 with features of antenatal asphyxia
9	M	27	1100	Apnoea of prematurity	18	Prematurity (27/40)	Very Low Birth Weight; jaundice; hyperglycaemia; first-born twin; neutropenia; thrombocytopenia; maternal PET and uncontrolled hypertension; bilateral hydroceles; hyponatraemia; Respiratory Distress Syndrome	Platelet count resolved after 2 weeks
12	F	35	1775	Not recorded	24	Preterm: 35/40	Physiological jaundice; thrombocytopenia; "flat at birth - meconium suctioned below cords"; LBW, symmetrical IUGR	Repeat platelet count was normal after 2 days
22	M	25	980	Admitted due to prematurity, respiratory distress and possible sepsis	37	Early neonatal death due to extreme prematurity	Final diagnosis: antepartum asphyxia following idiopathic ICH leading to hypovolaemia	Ultrasound head: Severe intracranial and intraparenchymal bleeding
23	M	34	1580	Preterm with respiratory distress.	38	Preterm: 34/40	Hypospadias; thrombocytopenia; feed intolerance; hypoglycaemia; RDS; IUGR; LBW	Platelet count resolved after 10 days.
28	F	35	1070	Admitted due to severe IUGR.	42	Preterm:35/40	Right inguinal hernia; RDS intubated; severe SGA 1070g; hypoglycaemia; hyponatraemia; bilateral grade 1 IVH; CMV positive; conjugated hyperbilirubinaemia; anaemia; renal impairment	Repeat platelet counts fluctuated between thrombocytopenia from day 1 to 2 weeks and then thrombocytosis after day 19 and eventually normalised after day 26.

No	Sex	GA	BW (g)	Reasons for Admissions	Platelet Count	Primary Diagnosis	Secondary Diagnosis	Outcome and record of resolution of thrombocytopenia
1	M	39	3350	Admitted due to thrombocytopenia	5	NAIT	Not recorded	Repeat platelet count was normal after 2 weeks
2	M	Term	2720	Normal new born examination except for petechial rash.	6	NAIT	Symmetrical IUGR	Repeat platelet count was normal after 1 day
3	F	39	3620	Not recorded	7	Not recorded	Not recorded	Repeat sample was normal after 2 hours
4	M	39	3600	Known family history of NAIT	7	Sibling of #1	Not recorded	Platelet count resolved after 2 weeks
6	F	38	Not recorded	Not recorded	9	Not recorded	Not recorded	Repeat platelet count remained decreased
7	F	40	Not recorded	Not recorded	11	Not recorded	Not recorded	Repeat platelet count was normal after 5 hours
8	F	38	Not recorded	Not recorded	13	Not recorded	Not recorded	Not recorded
10	F	39	Not recorded	Not recorded	18	Not recorded	Not recorded	Repeat platelet count was normal after 4 hours

11	F	38	5500	Not recorded	22	Not recorded	Not recorded	Stillbirth due to diabetes and pre-eclampsia
13	F	37	3220	Establish breast feeding	27	HDN	Bilateral talipes; pathological jaundice; erythropoiesis; thrombocytopenia; anaemia; hepatomegaly; cardiomegaly; PPHN	Repeat platelet count was normal after 2 weeks
14	M	39	2940	Infant re-admitted with low cord blood platelets count.	27	NAIT	Maternal group B step positive	Repeat platelet count was normal after 1 day
15	M	Term	2400	Not recorded	29	Mild IUGR	Thrombocytopenia in neonatal period	Platelet count resolved after 3 weeks
16	F	40	Not recorded	Not recorded	30	Not recorded	Not recorded	Repeat count resolved after 3 weeks
17	M	39	3300	Admitted due to thrombocytopenia	32	NAIT	Known maternal ITP & splenectomy	Platelet count remained decreased
18	F	38	3130	Not recorded	32	Not recorded	Not recorded	Probable NAIT with VSD. Platelet count resolved after 10 days
19	M	38	2465	Hypothermia & hypoglycaemia	35	Gram negative septicaemia	Thrombocytopenia; hypotension; metabolic acidosis; multi-organ failure; DIC; hyponatremia; hypoglycaemia; SGA	Baby died after 4 days of sepsis.
20	M	40	Not recorded	Not recorded	35	Not recorded	Not recorded	Stillbirth
21	M	40	Not recorded	Not recorded	36	Not recorded	Not recorded	Platelet count resolved after 4 days

24	M	41	Not recorded	Not recorded	40	Not recorded	Not recorded	Repeat sample after 3 days showed normal platelet count
25	M	38	Not recorded	Not recorded	40	Not recorded	Not recorded	Platelet count was normal after 1 day
26	M	37	2135	Not recorded	41	IUGR	Pseudo-TORCH syndrome, abnormal head scan, thrombocytopenia	Possible NAIT, but excluded serologically.
27	M	39	3565	Jaundice and Seizures	41	Bilirubin encephalopathy/kernicterus	Healing fracture right clavicle; thrombocytopenia; gastro-oesophageal reflux; increased tone; irritability; seizures	Repeat platelet count was normal after 18 days
29	M	40	Not recorded	Not recorded	42	Not recorded	Not recorded	Repeat platelet count: resolved after 4 hours (188)
30	F	40	2710	Not recorded	42	Not recorded	Not recorded	No repeat sample provided
31	M	39	3000	Not recorded	46	Not recorded	Suggested alpha thalassaemia with low MCV (99fL)	No repeat sample provided

NAIT: neonatal alloimmune thrombocytopenia; TORCH: Toxoplasma gondii, other viruses (HIV, measles, and so on), rubella (German measles), cytomegalovirus, and herpes simplex; SGA: smaller than gestation age; IVH: intraventricular haemorrhage, CMV: cytomegalovirus; PPHN: Persistent pulmonary hypertension of the newborn; HDN: haemolytic disease of the newborn; ITP: Idiopathic thrombocytopenic purpura; IUGR: intrauterine growth retardation; DIC: disseminated intravascular coagulation; VSD: ventricular septal defect, PET: Pre-eclamptic toxaemia; RDS: respiratory distress syndrome, LBW: low birth weight; MCV: mean cell volume

5.4 Clinical state of thrombocytopenic infants

Babies were categorised as “asymptomatic” if they were not admitted or did not have a medical history other than thrombocytopenia, and “symptomatic” if they were admitted with one or more medical conditions as well as thrombocytopenia. Both asymptomatic and symptomatic infants were further classified as having severe, moderate or mild thrombocytopenia.

Amongst the 794 thrombocytopenic babies included in the audit, 67% were classified as asymptomatic and 33% symptomatic. Preterm infants accounted for 143 (18%), of which 4.9% were asymptomatic and 13.1% were symptomatic. Term infants comprised a further 641(80.8%) cases, of which 61.0% were asymptomatic and 19.8% were symptomatic. Eight (1%) babies were post term, of which 0.1% were symptomatic and 0.9% were asymptomatic. Finally, 0.2% of the infants with unknown gestational age were asymptomatic (Table 5.2, Table 5.6, Table 5.7 and Figure 5.2).

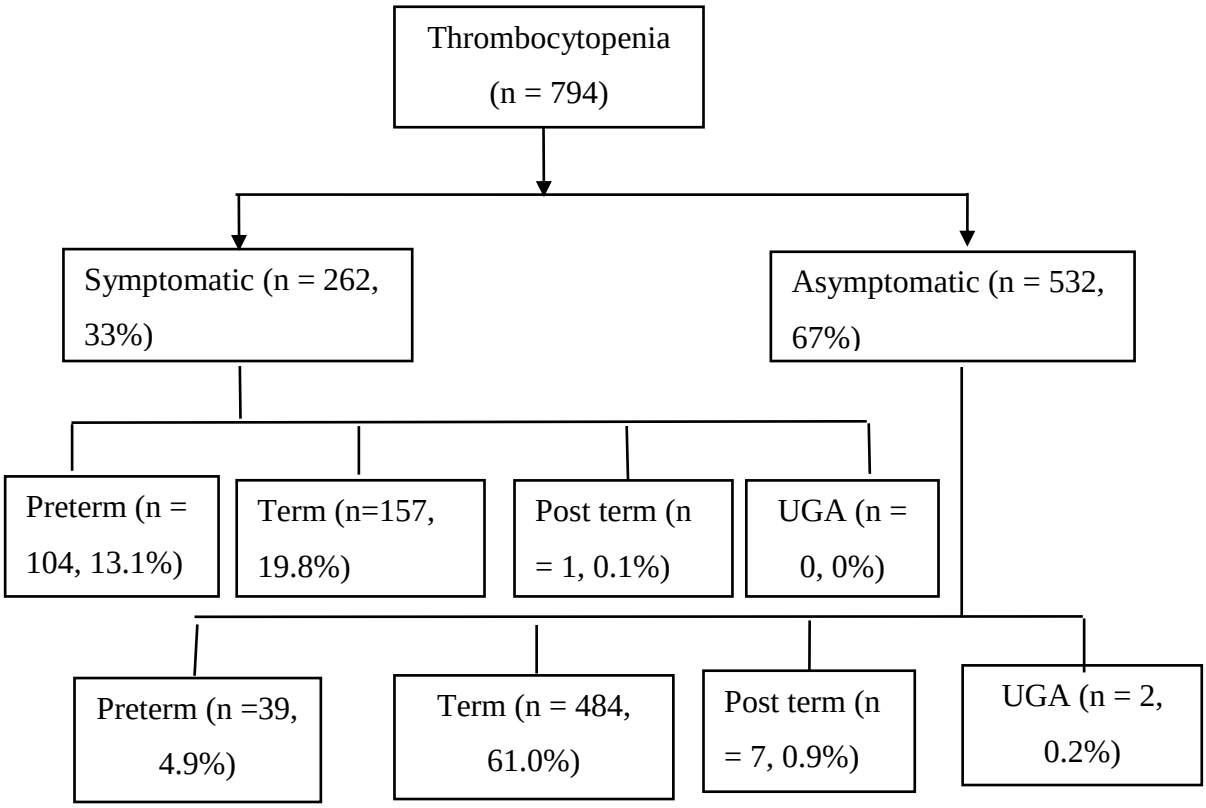

Figure 5.2. Flow chart of thrombocytopenic infants according to gestational age and clinical status.

5.4.1 Symptomatic Thrombocytopenic Infants

Preterm

Amongst the 262 symptomatic infants, 104 (39.7%) were preterm. Of these, 2.3% had severe thrombocytopenia (1.5% males and 0.8% females), 8.4% had moderate thrombocytopenia (4.6% males and 3.8% females), and 29% had mild (14.9% males and 14.1% females) (Table 5.6 and Figure 5.2).

Term

There were 157 (59.9%) symptomatic term infants, of which 4.9% had severe thrombocytopenia (3.8% males and 1.1% females), 11% had moderate (5.7% males and 5.3% females), and 43.9% had mild (27.5% males and 16.4% females) (Table 5.6 and Figure 5.2).

Post term

The thrombocytopenia in the single male post term infant was moderate (0.4%) (Table 5.6 and Figure 5.2).

Table 5.6. Symptomatic thrombocytopenic infants according to severity, gender and gestational age

	Symptomatic n = 262, 33.0%													
	Severe (n = 19, 7.2%)				**Moderate (n = 51, 19.5%)**				**Mild (n = 192, 73.3%)**				**Totals (n = 262)**	
Gestational age	**Male**	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**M**	**%**	**F**	**%**	**Total**	**%**
Preterm	4	1.5	2	0.8	12	4.6	10	3.8	39	14.9	37	14.1	104	39.7
Term	10	3.8	3	1.1	15	5.7	14	5.3	72	27.5	43	16.4	157	59.9
Post Term	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.4	1	0.4
Unknown gestational age	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	**14**	5.3	**5**	1.9	**27**	10.3	**24**	9.2	**111**	42.4	**81**	30.9	**262**	**100.0**

5.4.2 Asymptomatic Thrombocytopenic infants

Amongst the 532-asymptomatic infants, 2.3% had severe thrombocytopenia (1.0% males and 1.3% females), 10.9% had moderate (5.8% males and 5.1% females), 86.8% had mild (49.2% males and 37.6% females) (Table 5.7 and Figure 5.2).

Preterm

Thirty-nine (7.3%) of the asymptomatic thrombocytopenic infants were preterm. None had severe thrombocytopenia, 0.8% had moderate (0.4 % males and 0.4% females), and 6.6% had mild (3.8% males and 2.8% females) (Table 5.7 and Figure 5.2).

Term

Of the 484 (91.0%) asymptomatic term infants, 2.3% had severe thrombocytopenia (1.0% males and 1.3% females), 10.0% had moderate (5.3% males and 4.7% females) and 78.7% had mild (44.7% males and 34.0% females) (Table 5.7 and Figure 5.2).

According to clinical details and history, one asymptomatic term infant (number 209) was readmitted 5 days after birth when the mother noticed a petechial rash on her baby. A repeat platelet count at that time showed severe thrombocytopenia. The diagnosis was not clear, but the working diagnosis was NAIT. The parents were not tested and there is no record of any siblings.

Post term

Of the seven (1.3%) asymptomatic post term thrombocytopenic infants, none had severe thrombocytopenia, 0.1% male had moderate and none were females, and 1.2% had mild (0.8% males and 0.4% females) (Table 5.7 and Figure 5.2).

Unknown gestation age

Of the two (0.4%), asymptomatic thrombocytopenic infants with unknown gestation age both were females and both had mild thrombocytopenia (Table 5.7 and Figure 5.2).

Table 5.7. Thrombocytopenic asymptomatic infants according to severity, gender and gestational age

Gestation age	**Asymptomatic Thrombocytopenic infants (n = 532, 67.0%)**													
	Severe (n =12)				**Moderate (n = 58)**				**Mild (n = 462)**				**Totals (n = 532)**	
	Male	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**		**%**
Preterm	0	0.0	0	0.0	2	0.4	2	0.4	20	3.7	15	2.8	39	7.3
Term	5	1.0	7	1.3	28	5.3	25	4.7	238	44.7	181	34.0	484	91.0
Post	0	0.0	0	0.0	1	0.1	0	0.0	4	0.8	2	0.4	7	1.3
Unknown gestation age	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.4	2	0.4
Total	**5**	1.0	**7**	1.3	**31**	5.8	**27**	5.1	**262**	49.2	**200**	37.6	**532**	**100.0**

5.5 Asymptomatic thrombocytopenic infants: maternal history and siblings.

5.5.1 Mothers

Five hundred-and-eight mothers of asymptomatic thrombocytopenic infants had normal platelet counts. Fourteen mothers had low platelet counts and nine were found to have various additional medical problems including seven with idiopathic thrombocytopenic purpura *(*ITP*),* and two with gestational diabetes mellitus (GDM). The remaining five had no recorded explanation for the thrombocytopenia. Six mothers had no record of platelet count, and four had no records at all (Figure 5.3).

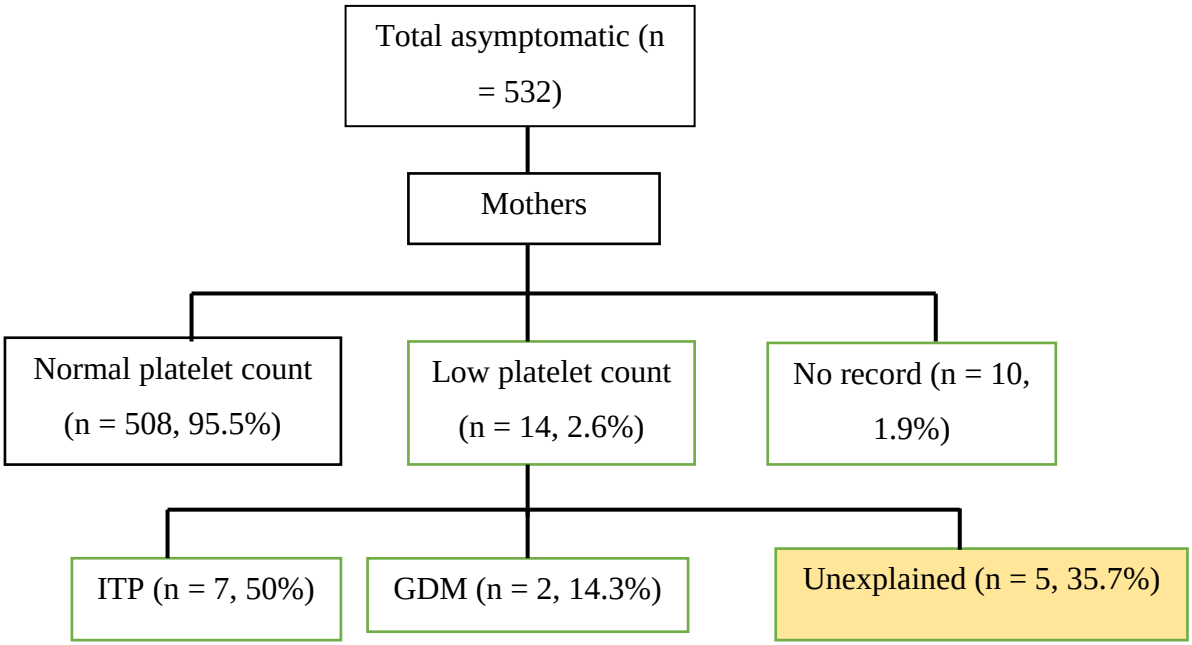

Figure 5.3. Presence of thrombocytopenia in mothers of asymptomatic infants

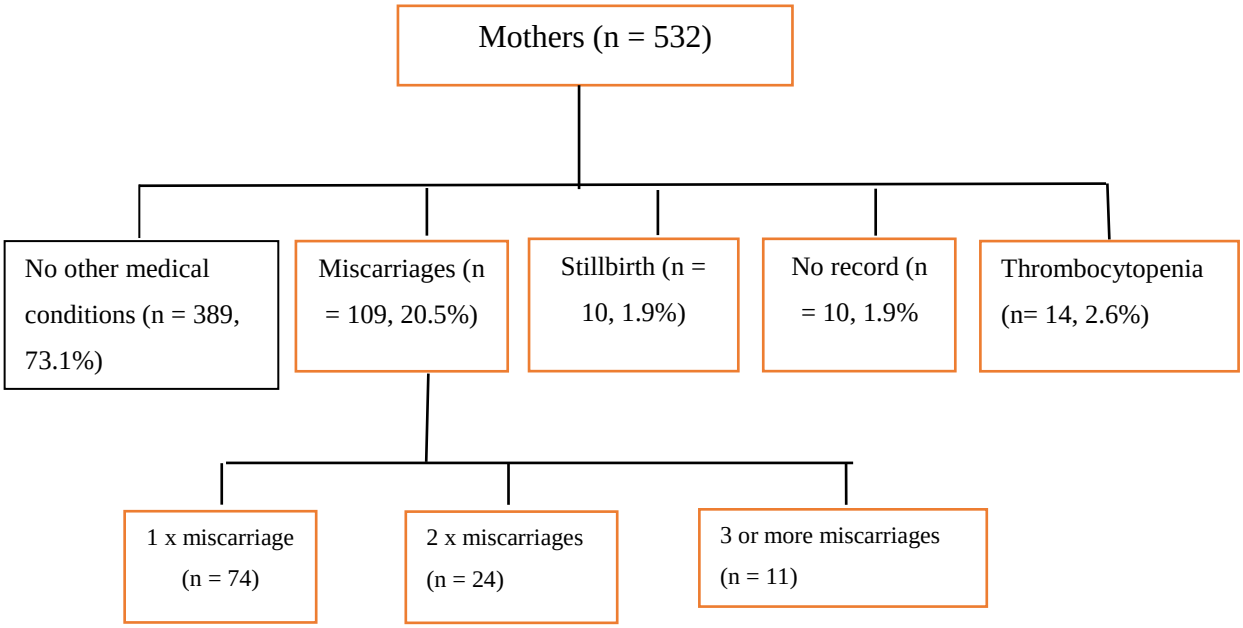

Figure 5.4. Occurrence of miscarriage and stillbirths amongst mothers of asymptomatic thrombocytopenic infants

Table 5.8. Medical conditions and clinical details of asymptomatic infants' mothers.

Maternal conditions that may explain infant's low platelet count	**Total**	**Percentage (%)**	**Maternal conditions that are less likely to cause thrombocytopenia in infants**	**Total**	**Percentage (%)**
Type 2 diabetes mellitus	12	2.3	Miscarriage	109	20.5
Gestational diabetes mellitus	11	2.1	Termination of pregnancy (TOP)	23	4.3
Stillbirths	10	1.9	Ectopic pregnancy	2	0.4
ITP	7	1.3	Substance abuse	2	0.4
PET	3	0.6	Alpha thalassaemia carrier	2	0.4
Maternal history of giving birth to IUGR infant	2	0.4	Beta thalassaemia carrier	2	0.4
SLE	1	0.2	Unplanned pregnancy	1	0.2
			Abortion	1	0.2
			Iron deficiency	1	0.2
			Depression	1	0.2
			Pyelonephritis	1	0.2
			Moved overseas	1	0.2
			No record	4	0.8
Total	**46**	**8.6**		**150**	**28.2**

Amongst the mothers of asymptomatic thrombocytopenic infants, 73.1% had no medical conditions or clinical details recorded; 20.5% had at least one miscarriage,2.6% had thrombocytopenia, 1.9% had stillbirths (i.e. there were ten recorded cases of still births amongst the asymptomatic infants' mothers) and 1.9% had no record on the database. Table 5.8 shows the percentages of some of the maternal conditions that are probable causes of thrombocytopenia in the infants and conditions that are unlikely to cause neonatal thrombocytopenia (Carr et al., 2017; Gupta, Mathai, & Kanitkar, 2011).

5.5.2 Infant siblings

Of the 532 asymptomatic infants, 373 (70.1%) had siblings, 29.1% had no siblings, and 0.8% had no record of the mother and siblings (Figure 5.5).

Of the 373 infants with siblings, 47.5% had one sibling, 21.7% had two, 10.7% had three siblings, and 7.8% had more than four siblings (5.4% had four, 1.9% had five and 0.5% had six). The remaining 12.3% of infants had siblings who had no recorded platelet counts (Figure 5.5).

Sixteen infants (4.9%) had siblings with a documented thrombocytopenia. 2.4% of these infants had one sibling with a documented thrombocytopenia, 1.8% infants had two, and 0.6% had four or more siblings with thrombocytopenia (one infant had five thrombocytopenic siblings and one infant had six) (Figure 5.5).

About 68.8% of the causes of thrombocytopenia were identified and 31.2% were not. (Figure 5.5).

Of the eight (2.4%) infants that had one sibling with thrombocytopenia, the causes identified include three infants with maternal ITP, one infant with a mother who had a history of previous IUGR and gestational diabetes mellitus, and one with schizophrenia. Three infants had no recorded (Figure 5.5).

Of the six (1.8%) infants who had two siblings with thrombocytopenia, the causes identified include two infants with maternal ITP, one with type 2 diabetes mellitus and anti – E antibodies in the mother, one infant with type 2 diabetes mellitus in the mother, and one infant was a twin with twin – to - twin transfusion syndrome. There was also one infant whose mother had had three miscarriages (Figure 5.5).

Of the two (0.6%) infants with five and six siblings with thrombocytopenia, this was thought to be due to maternal ITP (Figure 5.5).

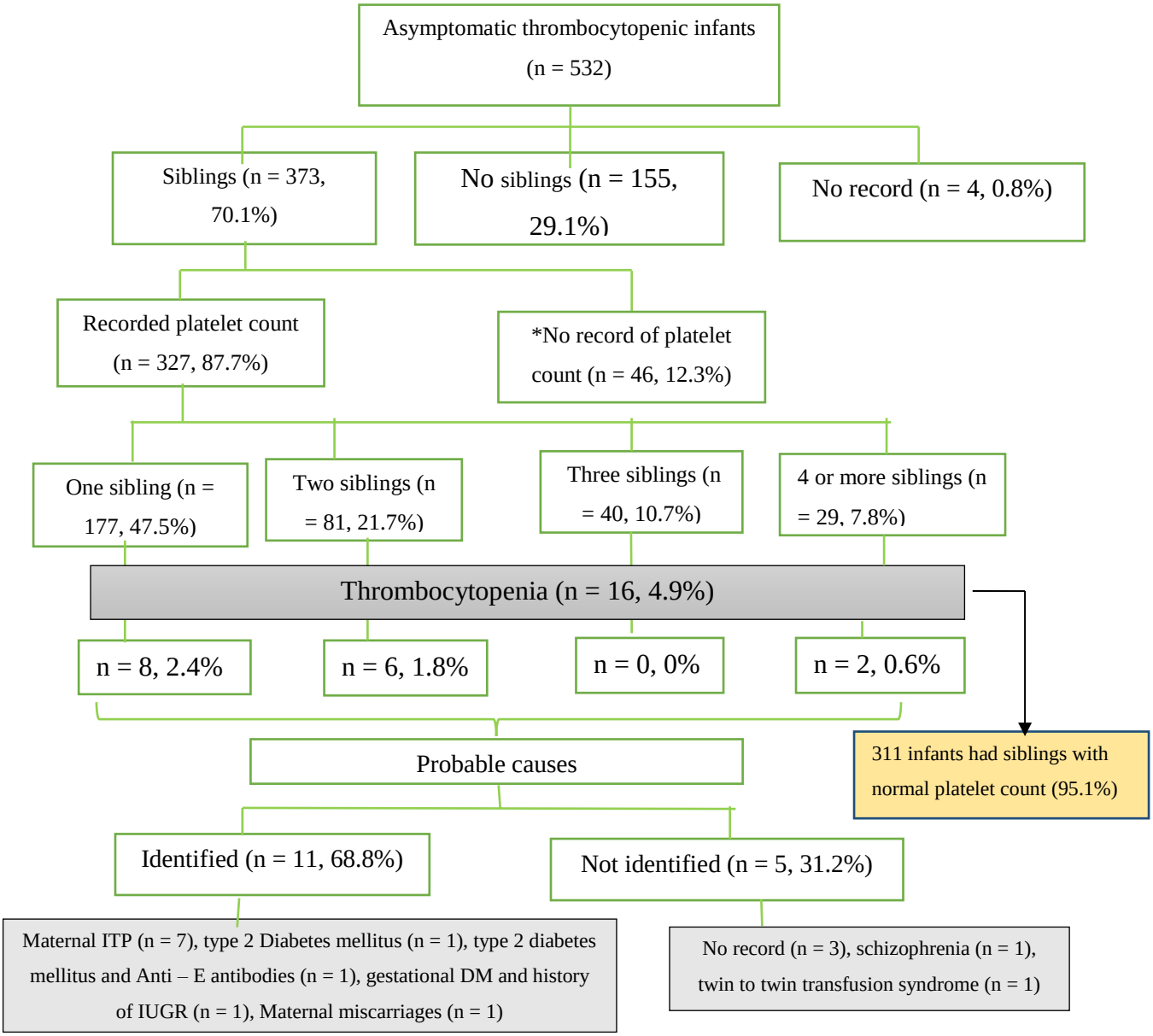

Figure 5.5. Thrombocytopenia in siblings of asymptomatic thrombocytopenic infants

* 46 siblings (12.3%) who did not have a record of the platelet count samples which were either clotted or clumped,

5.5.3 Relative risk

= (16/327) ÷ (799/66109)

= (0.04893) ÷ (0.01209)

= 4.0

95% Confidence Interval (CI) (2.41, 6.33).

A relative risk of greater than 1, as here, indicates that there is a higher risk (4 times as likely) of siblings developing thrombocytopenia than their infant sibling counterparts. The confidence interval of RR of thrombocytopenia in siblings compared to their infant sibling is between 2.41 and 6.33.

5.5.4 Ethnicity

Of the 532 mothers of asymptomatic thrombocytopenic infants, 109 were recorded as Samoan, 89 were New Zealand Maori, 89 were New Zealand Europeans, 67 were Indians, 53 were Tongan, 29 were Cook Islands Maori, 27 were other Asians, 14 were other European, 13 were Chinese, 12 were Fijians, nine were Niuean, five were European, five were Middle Eastern, four were "others", four had no ethnicity recorded, and three were other Pacific Islands.

The mother's ethnicities were then reclassified into groups which share specific platelet antigens at similar frequencies (www.adhb.govt.nz, n.d). Thus, there were 40.4% mothers of Pacific origin, 20.3% European, 20.1% Asian, 16.7% New Zealand Maori, 0.9% from the Middle East and 1.5% were from diverse groups termed "others".

Table 5.9. Ethnicity of mothers of asymptomatic thrombocytopenic infants

Ethnicity	Total	Percentage (%)
Samoa	109	20.5
New Zealand Maori	89	16.7
New Zealand European	89	16.7
Indian	67	12.6
Tongan	53	10.0
Cook Island Maori	29	5.4
Other Asians	27	5.1
Other European	14	2.6
Chinese	13	2.4
Fiji	12	2.3
Niuean	9	1.7
European	5	0.9
Middle East	5	0.9
Other	4	0.8
Other Pacific Islands	3	0.6
No record of ethnicity	4	0.8

Ethnicity	Total	Percentage (%)	CMDHB report 2016
Pacific Islands	215	40.4	28.0
Europeans	108	20.3	26.3
Asians	107	20.1	25.9
New Zealand Maori	89	16.7	19.8
Others	8	1.5	0
Middle East	5	1.0	0

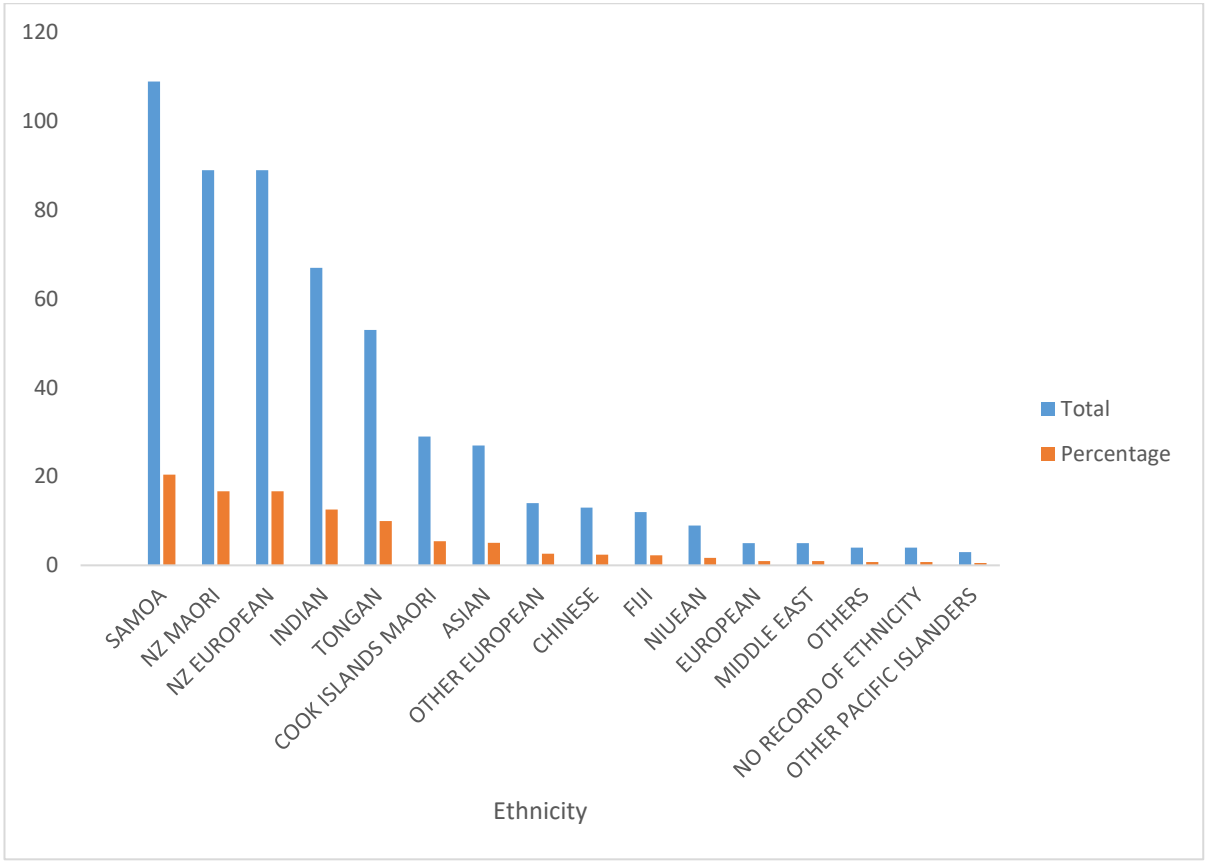

Figure 5.6. Ethnicity of mothers of asymptomatic thrombocytopenic infants born at Middlemore Hospital between 2005 and 2016.

5.6 Symptomatic thrombocytopenic infants

5.6.1 Clinical reasons for admissions.

Of the 794 thrombocytopenic infants initially identified, 31.5% were admitted to the neonatal unit immediately after birth. This number comprised seventeen (6.8%) of the 31 babies with severe thrombocytopenia, 47 (18.8%) of the 109 with moderate thrombocytopenia and 186 (74.4%) of the 654 who had mild thrombocytopenia. The four infants who have no record of gender, gestational age and birth weight on the Concerto database, but who were all thrombocytopenic, presumably were not admitted (Table 5.10 and Figure 5.7).

Table 5.10. Total number of hospitalised symptomatic thrombocytopenic infants by severity, gestational age and gender

Gestation age	Severe (n = 17)				Moderate (n = 47)				Mild (n = 186)				Total					
	Male	%	Female	%	Male	%	Female	%	Male	%	Female	%	Males	%	Females	%	Total	%
Preterm	4	1.6	2	0.8	10	4.0	9	3.6	35	14.0	34	13.6	49	19.6	45	18	94	37.6
Term	9	3.6	1	0.4	11	4.4	11	4.4	69	27.6	40	16.0	89	35.6	52	20.8	141	56.4
Post Term	0	0.0	0	0.0	0	0.0	1	0.4	0	0.0	1	0.4	0	0	2	0.8	2	0.8
Unknown GA	0	0.0	1	0.4	5	2.0	0	0.0	3	1.2	4	1.6	8	3.2	5	2	13	5.2
Total	**13**	5.2	**4**	1.6	**26**	10.4	**21**	8.4	**107**	42.8	**79**	31.6	146	58.4	104	41.6	250	31.5

Table 5.11. Thrombocytopenic infants not admitted

	Severe (n = 14)				Moderate (n = 62)				Mild (n = 468)				Total (n = 544)					
	Male	**%**	Female	**%**	Male	**%**	Female	**%**	Male	**%**	Female	**%**	Male	**%**	Female	**%**	Total	**%**
Total Symptomatic not admitted	1	**0.2**	1	**0.2**	2	**0.4**	2	**0.4**	4	**0.7**	2	**0.4**	7	**1.3**	5	**0.9**	12	**2.2**
Asymptomatic Infants	5	**0.9**	7	**1.3**	31	**5.7**	27	**5.0**	262	**48.2**	200	**36.8**	298	**54.8**	234	**43.0**	532	**97.8**
Total	**6**	**1.1**	**8**	**1.5**	**33**	**6.1**	**29**	**5.3**	**266**	**48.9**	**202**	**37.1**	305	**56.1**	239	**43.9**	544	**68.5**

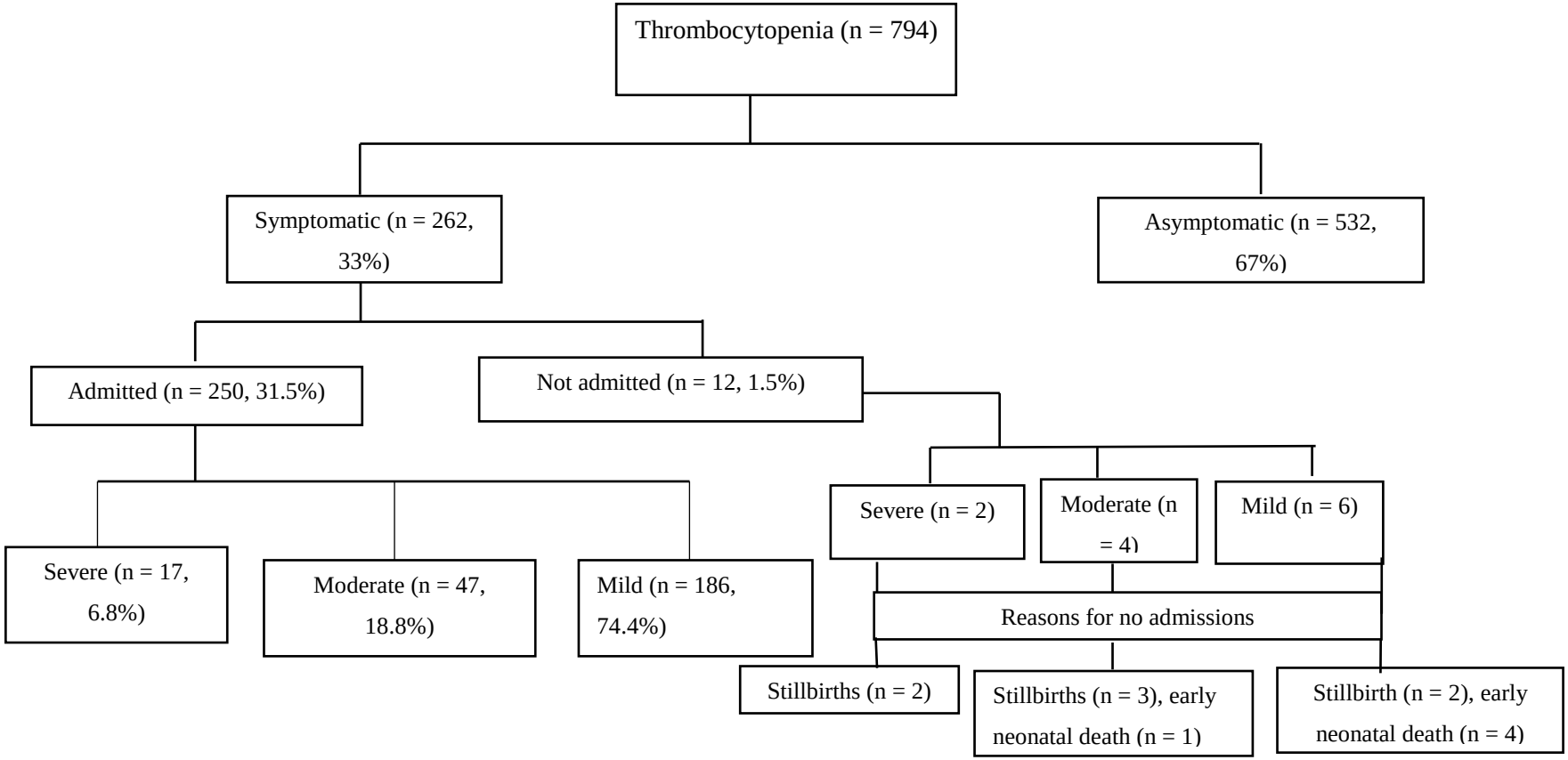

Figure 5.7. Flow diagram of infants admitted.

All except 12 (1.5%) of the symptomatic thrombocytopenic infants were admitted to hospital. These 12 had all died before or soon after birth. Thus, seven were recorded as stillbirths and five as early neonatal deaths (Table 5.11 and Figure 5.7).

The clinical summaries of infants admitted to Middlemore Hospital Neonatal Intensive Care Unit (NICU) included reasons for admission, and the primary and secondary diagnoses in each case.

The reasons for admission provide information on the patient's condition at presentation. The primary diagnosis is the main diagnosis established for the patient after being admitted to the hospital, and the main indication for appropriate healthcare. The secondary diagnosis refers to other diagnoses and conditions which either coexisted at the time of admission, or which may have developed afterwards. These can affect the plan of treatment for the patient. These clinical reasons and diagnoses are shown in Table 5.12 and Table 5.13.

Table 5.12. Diseases associated with thrombocytopenia in study infants

Clinical Conditions	Reasons for admission				Primary diagnosis				Secondary diagnosis				Total clinical conditions
	Preterm	Term	Post term	Total	Preterm	Term	Post term	Total	Preterm	Term	Post term	Total	
RDS	46	58	1	105	7	25	0	**32**	48	32	0	**80**	**217**
Hypoglycaemia	4	45	0	49	1	17	0	**18**	36	53	0	**89**	**156**
Preterm	49	0	0	49	77	0	0	**77**	5	0	0	**5**	**131**
Thrombocytopenia	0	4	0	4	1	1	0	**2**	43	46	0	**89**	**95**
LBW	10	4	0	14	0	0	0	**0**	55	10	0	**65**	**79**
IUGR	10	4	0	14	0	10	0	**10**	20	22	3	**45**	**69**
Meconium exposure	0	12	1	13	0	8	0	**8**	7	34	0	**41**	**62**
Jaundice	0	3	0	3	0	1	0	**1**	36	21	1	**58**	**62**
Sepsis	3	3	1	7	1	4	0	**5**	10	2	0	**12**	**24**
Apnoea of prematurity	2	0	0	2	0	0	0	**0**	13	0	0	**13**	**15**
Seizure	0	3	0	3	0	0	0	**0**	3	8	0	**11**	**14**
LGA	0	1	0	1	0	1	0	**1**	2	9	0	**11**	**13**
Downs syndrome	0	4	0	4	1	7	0	**8**	0	0	0	**0**	**12**
TTN	0	1	0	1	1	6	1	**8**	1	1	0	**2**	**11**
Perinatal Asphyxia	1	2	0	3	2	3	0	**5**	0	1	0	**1**	**9**
NAIT	0	0	0	0	0	5	0	**5**	0	0	0	**0**	**5**

Key: IUGR: Intrauterine growth retardation; LGA: larger than gestation age; NAIT: neonatal alloimmune thrombocytopenia; TTN: transient tachypnoea of newborn, RDS: respiratory distress syndrome; LBW: low birth weight

Table 5.13. Summary of diseases associated with thrombocytopenia irrespective of gestational age

Diseases	Reasons for admission	Primary diagnosis	Secondary diagnosis	Total	Percentage
RDS	105	**32**	**80**	**217**	86.8
Hypoglycaemia	49	**18**	**89**	**156**	62.4
Preterm	49	**77**	**5**	**131**	52.4
Thrombocytopenia	4	**2**	**89**	**94**	37.6
LBW	14	**0**	**65**	**79**	31.6
IUGR	14	**10**	**45**	**69**	27.6
Meconium exposure	13	**8**	**41**	**62**	24.8
Jaundice	3	**1**	**58**	**62**	24.8
Sepsis	7	**5**	**12**	**23**	9.2
Apnoea of prematurity	2	**0**	**13**	**15**	6
Seizure	3	**0**	**11**	**14**	5.6
LGA	1	**1**	**11**	**13**	5.2
Downs syndrome	4	**8**	**0**	**12**	4.8
TTN	1	**8**	**2**	**11**	4.4
Perinatal Asphyxia	3	**5**	**1**	**8**	3.2
NAIT	0	**5**	**0**	**5**	2

Note: most infants were recorded as having more than one condition either on admission or at discharge.

Regardless of the gestational age and diagnosis, 78.6% had unexplained causes of the thrombocytopenia, 20.2% of the infants with thrombocytopenia were due to known causes, and 1.3% were due to NAIT (Table 5.13 and Figure 5.8 and Figure 5.9).

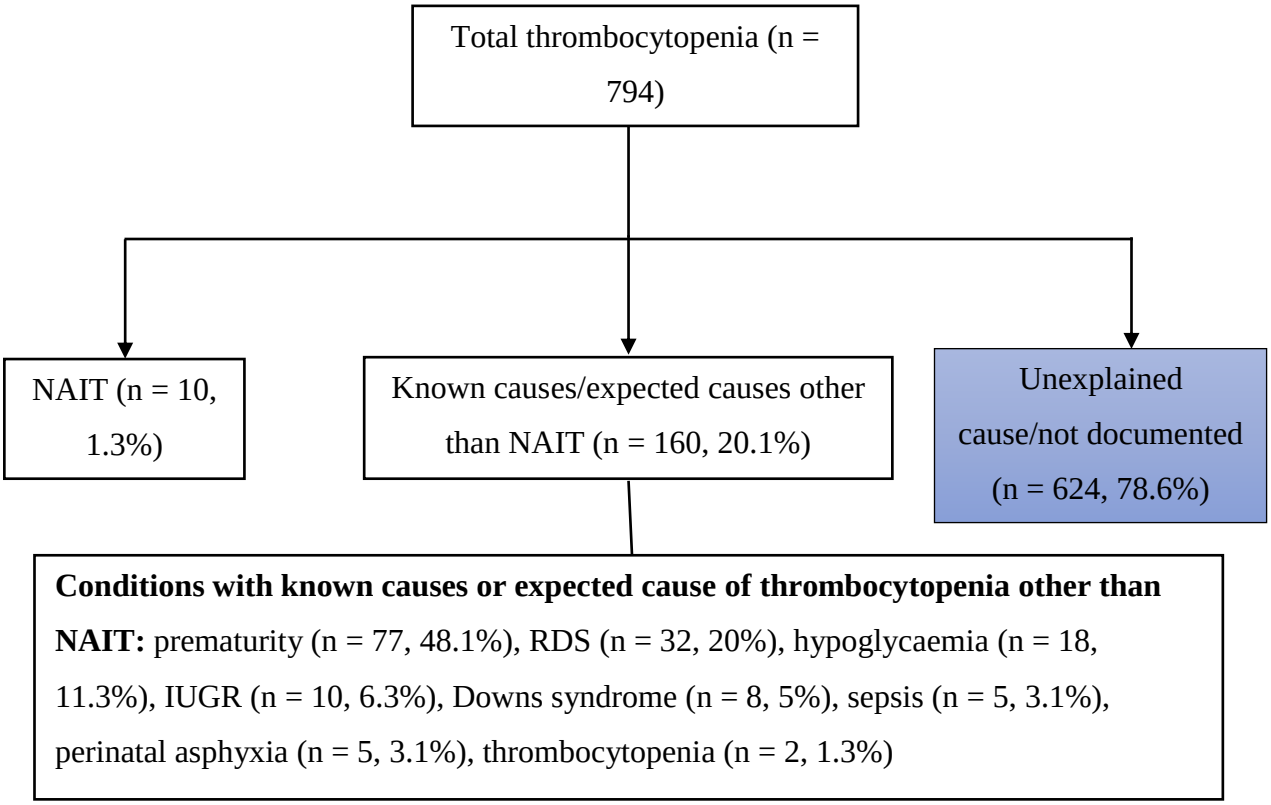

Figure 5.8. Explanations for cause of thrombocytopenia in study infants

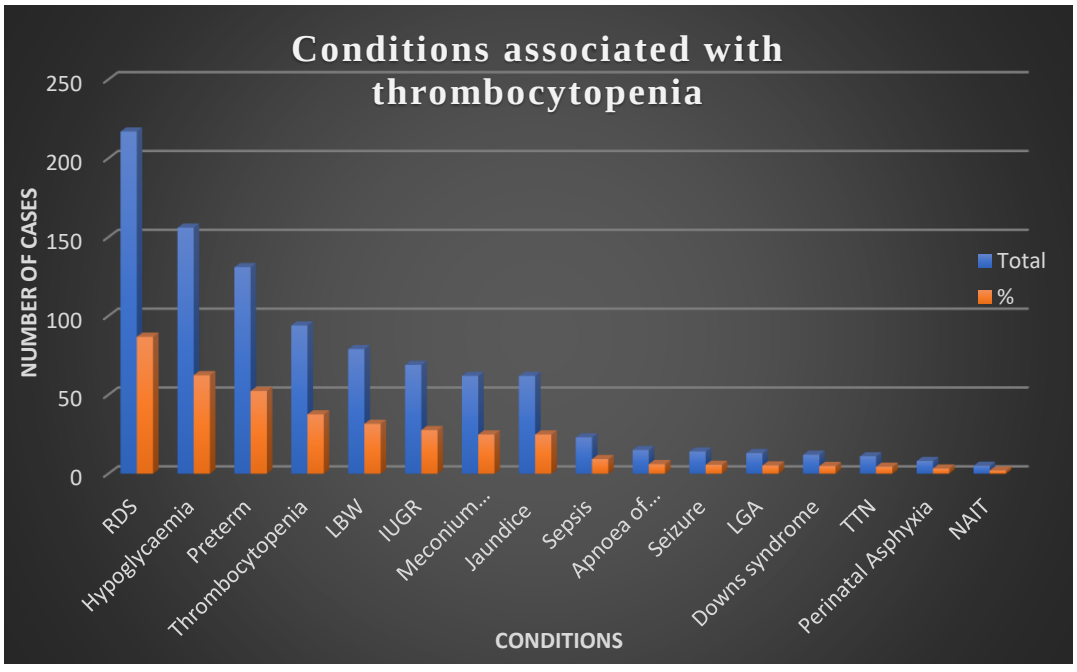

Figure 5.9. Conditions associated with thrombocytopenia in infants

5.6.2 Intracranial haemorrhage

Of the 794 thrombocytopenic infants, 11 cases of intracranial haemorrhage (ICH) were identified and all of them occurred in preterm infants. The repeat platelet count resolved in 9 of these. The remaining two infants died of intraventricular and intraparenchymal bleeding and prematurity. The gestational age amongst these babies ranged from 20 to 35 weeks (Table 5.14).

Table 5.14. Evidences of intracranial haemorrhage

					Results	Outcome
Infant number	**Sex**	**GA**	**BW**	**Platelet Count**	**X ray/Ultrasound**	
22	M	25	980	37	Severe intraventricular and intraparenchymal bleeding.	Infant died after seven hours
28	F	35	1070	42	Bilateral Grade 1 IVH	Repeat platelet count remained decreased for 3 weeks, then rose above the reference range after 25 days before it normalised.
35	F	28	1035	52	Grade 1 IVH Left	Maternal pre-eclampsia and platelet count resolved after three weeks
42	F	31	930	58	IVH: Grade 1 (left); Grade2 (right) - cystic changes	Platelet count resolved after 10 days.
71	M	33	2660	78	Intraventricular, parenchymal and pituitary haemorrhage	Platelet count resolved after 11 days. Possible twin to twin transfusion syndrome
73	M	33	1920	79	Bilateral grade III IVH	Platelet count resolved after 5 days
252	M	24	59	116	Grade 4 IVH Right side	Platelet count resolved after 5 days
326	F	28	1000	121	IVH Grade 2 Right	Platelet count resolved after 10 days
508	F	23	715	135	Left grade IV and right grade II intraventricular haemorrhage	Infant died due to extreme prematurity. Other illnesses: bronchopulmonary dysplasia, E. coli sepsis, renal failure - hyperkalaemia, suspected necrotising enterocolitis, PDA - treated and corrected, hydrocephalus and hyperglycaemia on insulin
605	F	30	1449	141	IVH grade II	Platelet count resolved after two weeks
643	F	27	1070	143	Grade 1 IVH on the left	Platelet count resolved after one day

IVH: intraventricular haemorrhage; PDA: patent ductus arteriosus; GA: gestation age, BW: birth weight

5.6.3 Stillbirths and Neonatal deaths

Of 794 thrombocytopenic infants, a total of 23 infants were recorded as stillbirths and neonatal deaths over the ten – year study period. The causes identified include seven stillbirths with one due to congenital abnormality, one with spontaneous premature birth with chorioamnionitis, one with pre – eclampsia and antepartum haemorrhage and four without recorded cause. Sixteen neonatal deaths were documented, with four cases of extreme prematurity, four with perinatal asphyxia, two with congenital abnormality, two with hypoxic ischaemic encephalopathy (HIE), one with sepsis, one due to a seizure, one with intracranial haemorrhage, and one had a feto – maternal haemorrhage.

Most of the cases were preterm infants with 15 cases in the gestational age range of 21 to 36 weeks. The eight other neonatal deaths all occurred at term (Table 5.15).

Table 5.15. Total number of stillbirths and neonatal death recorded over ten years.

Infant no.	Sex	GA	BW	Platelet Count	Outcome	Cause of Stillbirth/Neonatal death
5	M	36	3150	8	Neonatal death	Neonatal death after Day 3 with features of antenatal asphyxia - unknown aetiology.
11	F	38	5500	22	Stillbirth	Post mortem declined
20	M	40	3835	35	Stillbirth	Post mortem declined
22	M	25	980	37	Neonatal death due to extremely preterm birth. X-ray: severe intraventricular and intraparenchymal bleeding	Unknown. Final diagnosis: antepartum asphyxia following idiopathic intracranial haemorrhage leading to hypovolaemia
34	F	41	4160	51	Hypoxic peripartum death: Neonatal death	Perinatal asphyxia
43	F	38	Not recorded	58	Stillbirth	Post mortem declined
44	F	33	Not recorded	59	Neonatal death	Baby died after 3 days of sepsis from congenital listeriosis.
51	F	21	260	62	Stillbirth	Cause of death: congenital abnormality, chromosomal
61	M	23	815	73	Stillbirth	Post mortem declined
79	M	36	108	81	Neonatal death	Preterm with twin- to- twin transfusion
120	F	37	2200	94	Neonatal death	Neonatal death due to tuberose sclerosis as diagnosed on echocardiogram/magnetic resonance imaging (MRI).
137	M	22	660	98	Neonatal death	Antepartum haemorrhage of undetermined origin with extreme prematurity. Post mortem declined.
156	M	38	3890	102	Neonatal death	Baby died after 2 days of hypoxic ischaemic encephalopathy grade II and multiple organ failure. Perinatal asphyxia

168	F	23	460	105	Neonatal death	Neonatal death due to extreme prematurity
221	M	34	2100	112	Neonatal death	Mostly likely cause: perinatal asphyxia. Hb Barts detected
361	F	21	390	124	Stillbirth	Spontaneous preterm with chorioamnionitis
492	F	36	Not recorded	134	Neonatal death	Cause of death: anaemia from severe feto - maternal haemorrhage and uncontrollable seizures
508	F	23	715	135	NND due to extreme prematurity. X ray: Left grade IV and right grade II intraventricular haemorrhage.	Cause of death due to extreme prematurity. Other illnesses: bronchopulmonary dysplasia, E. coli sepsis, renal failure - hyperkalaemia,suspected necrotising enterocolitis, PDA - treated and corrected, hydrocephalus, hyperglycaemia on insulin
535	F	23	640	137	Neonatal death	Extreme prematurity. Post mortem declined
660	M	Term	Not recorded	143	Neonatal death	Thanatotropic dysplasia.
690	M	35	2110	145	Stillbirth	Post mortem declined. Cause of death: pre – eclampsia and antepartum haemorrhage
724	F	34.4	1370	146	Neonatal death	Trisomy 18
728	F	33	1625	147	Neonatal death	Seizure

Key: PDA: Patent ductus arteriosus,

5.6.4 NAIT cases

The prime aim of this audit was to identify cases of possible NAIT in term infants. The selection criteria included thrombocytopenia, clinical details, family history of NAIT, previous siblings diagnosed with NAIT, radiology results showing intracranial haemorrhage and serological confirmation of NAIT. A total of 10 cases of NAIT were identified clinically and/or confirmed by serological tests. Table 5.16. Seven cases had severe thrombocytopenia, one moderate and two mild. Of the 10 reported cases, only three have records which confirm the diagnosis of NAIT. One case was a known case (confirmed by serological tests) and two cases were identified as siblings of a known infant who had been diagnosed with NAIT (Table 5.16).

Five of the 10 cases (numbers 1, 2, 4, 14 and 17) had a *primary diagnosis* stated as NAIT but only three (numbers 1, 2 and 4: two of which were siblings) were identified as confirmed NAIT. Another case (number 14) had no available record and one (number 17) was the infant of a mother with ITP (Table 5.16).

Cases of "suspected NAIT" were also extracted from the records. In these infants, NAIT was a tentative diagnosis based on clinical grounds but apparently not investigated.

There was one case of suspected NAIT (number 242) with mild thrombocytopenia, one (number 26) was initially considered as possible NAIT but later the thrombocytopenia was explained by confirmed congenital chromosomal abnormality, and one case (number 209) had no clear diagnosis but the working diagnosis was NAIT (mild thrombocytopenia). A further two cases were considered to be "probable" (number 18) and "presumed" (number 45) NAIT (Table 5.16)

Table 5.16: Infants with a working diagnosis of NAIT

Infant no.	Sex	Gestational age (weeks)	Birth-weight (g)	Clinical details	Ethnicity	Platelet count	ABO/ Rh	Diagnosis: H/W or Concerto		Other findings	Infants HPA	Maternal HPA	Paternal HPA
								Primary	Secondary diagnosis				
1	M	39	3350	Admitted to NICU due to thrombocyt openia.	Other European (England)	5	O +	NAIT	Not recorded	Sibling of Case # 4. Had twin siblings as well who also had severe thrombo-cytopenia	Not recorded	Serum Platelet – HPA -1a specific antibody detected Platelet family study results: mother's serum versus father's platelets: positive due to presence of serum platelet antibody. Platelet phenotyping results: mother: HPA-1a negative. ABO: O positive	Father: HPA-1a positive, homo-zygous HPA1a + positive
2	M	Term	2720	Normal new born examination except for petechial rash.	New Zealand European	6	A +	NAIT	Symmetrical IUGR		Baby HPA1a heterozyg ous	HPA genotype: HPA -1: 1b1b, 2: 2a2a, 3: 3a3b, 4: 4a4a, 5: 5a5a, 15:15a15a. Paternal/Materna l PLT Xmatch: Paternal PLT positive. ABO: O positive, G4PO (2 x TOP, 1 ectopic)	Father homo-zygous HPA1a +

4	M	39	3600	Marked thrombocyt openia secondary to known anti HPa1 antibodies in the mother.	Other European (England)	7	O +	NAIT	Not recorded	Sibling of Case # 1, and also had twin siblings.	Not recorded	Same as sibling #1	Father homo-zygous HPA1a +ve
14	M	39	2940	Infant re-admitted with severe thrombocyt openia	Other European (New Zealand)	27	O +	NAIT	Maternal group B step positive	Not recorded	Not recorded	Not recorded	Not recorded
17	M	39	3300	Admitted due to severe thrombocyt openia	Chinese	32	O +	NAIT	Known maternal ITP & splenectomy	Not recorded	Not recorded	Medical history: Known ITP, splenectomy, salpingectomy	Not recorded
18	F	38	3130	Not recorded	New Zealand European	32		Not recorded	Not recorded	VSD and probable NAIT	Not recorded	Not recorded	Not tested
26	M	37	2135	Not recorded	Indian	41	O +	Intrauterine growth restrictions (IUGR)	Pseudo-TORCH syndrome, abnormal head scan, thrombo-cytopenia	Bloods taken on parents for possible NAIT	Not NAIT	Not tested	Not tested
45	M	40	Not recorded	Admitted for repeat FBC	Cook Islands Maori	59	O +	Not recorded	Not recorded	Presumed NAIT with club foot	Not tested	No history in the mother of ITP, so it was assumed to be alloimmune thrombo cytopaenia:NAIT	Not tested
209	M	38	2910	Admitted for repeat FBC after 5	Cook Islands Maori	110	O +	Not recorded	Not recorded	The diagnosis is unclear, but the working	Not tested	No problems in pregnancy, no history of	Not tested

				days after noticing petechiae rash.						diagnosis is NAIT		bleeding problems in the family. Mother had normal platelet count.	
242	F	37	4815	Admitted because of respiratory distress and hypoglycaemia. Incidental finding of thrombocytopenia on admission.	Tongan	115	O +	Hypo-glycaemia	Not recorded	Not recorded	Suspected NAIT however unable to get blood sample from father	Type II diabetes	Not tested

HPA: Human platelet antigen, NAIT: neonatal alloimmune thrombocytopenia, HW: health ware, FBC: full blood count. ITP:Idiopathic thrombocytopenic purpura

5.7 Follow up

Where this was available, sequential platelet counts following the initial cord sample were recorded for all 794 thrombocytopenic infants in the study. This was termed follow – up. A total of 270 infants had at least one repeat platelet count, and of this 78.9% were symptomatic infants and 21.1% were asymptomatic at birth.

Of the 213 symptomatic infants, 41.3% were preterm, 58.7% were term.

Of the 57 asymptomatic infants, 7.0% were preterm, 89.4% were term, 1.8% case of post term, and 1.8% infants with no record of gestation age.

The results are tabulated in Table 5.17, Table 5.20 and Figure 5.10.

5.7.1 Symptomatic thrombocytopenic infants

Preterm Infants

Of the 104-symptomatic thrombocytopenic preterm infants, 41.3% had repeat a platelet count and 32.7% did not.

The 88 (41.3%) preterm infants who had a repeat platelet count included all six with severe thrombocytopenia, 18 of a possible 22 with moderate and 64 of a possible 76 with mild.

Amongst the remaining 6.1% symptomatic preterm infants who were not followed up 1.6% had a moderate, and 4.6% mild thrombocytopenia (Table 5.17 and Figure 5.10).

Term Infants

Of the 157 symptomatic term infants, 58.7% cases were followed up and 65.3% were not (Table 5.17 and Figure 5.10).

The 125 (47.7%) term thrombocytopenic infants who had a repeat platelet count included 11 of a possible 13 with severe thrombocytopenia, 23 of a possible 29 with moderate, and 91 of a possible 115 with mild.

Amongst the remaining 32 symptomatic term infants who were not followed up there were two with severe thrombocytopenia, five with moderate, and 25 with mild.

Post- term Infants

The only symptomatic post - term infant had no repeat platelet count. This infant had a mild thrombocytopenia at birth (Table 5.17 and Figure 5.10).

Table 5.17. Follow up of symptomatic thrombocytopenic infants with (A) or without (B) repeat platelet counts

A	Repeat platelet count (n = 213, 81.3)													
	Severe (n = 17, 8.0%)				**Moderate (n = 41, 19.2)**				**Mild (n = 155, 72.8%)**				**Total repeat**	
Gestational age (GA)	Male	%	Female	%	Male	%	Female	%	Male	%	Female	%	Total	%
Preterm	4	1.9	2	0.9	10	4.7	8	3.8	33	15.5	31	14.6	**88**	41.3
Term	10	4.7	1	0.5	14	6.6	9	4.2	57	26.8	34	16.0	**125**	58.7
Post term	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**0**	0.0
Unknown GA	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**0**	0.0
Total	**14**	6.6	**3**	1.4	**24**	11.3	**17**	8.0	**90**	42.3	**65**	30.5	**213**	100.0

B	**No repeat count (n = 49, 18.7%)**													
	Severe (n = 2, 4.0%)				**Moderate (n = 9, 18.4%)**				**Mild (n = 38, 77.6%)**				**Total no repeat**	
Gestation age	Male	%	Female	%	Male	%	Female	%	Male	%	Female	%	Total	%
Preterm	0	0.0	0	0.0	2	4.1	2	4.1	6	12.2	6	12.2	**16**	32.7
Term	1	2.0	1	2.0	2	4.1	3	6.1	15	30.6	10	20.4	**32**	65.3
Post term	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.0	**1**	2.0
Unknown GA	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**0**	0.0
Total	**1**	**2.0**	**1**	**2.0**	**4**	**8.2**	**5**	**10.2**	**21**	**42.8**	**17**	**34.7**	**49**	**100.0**

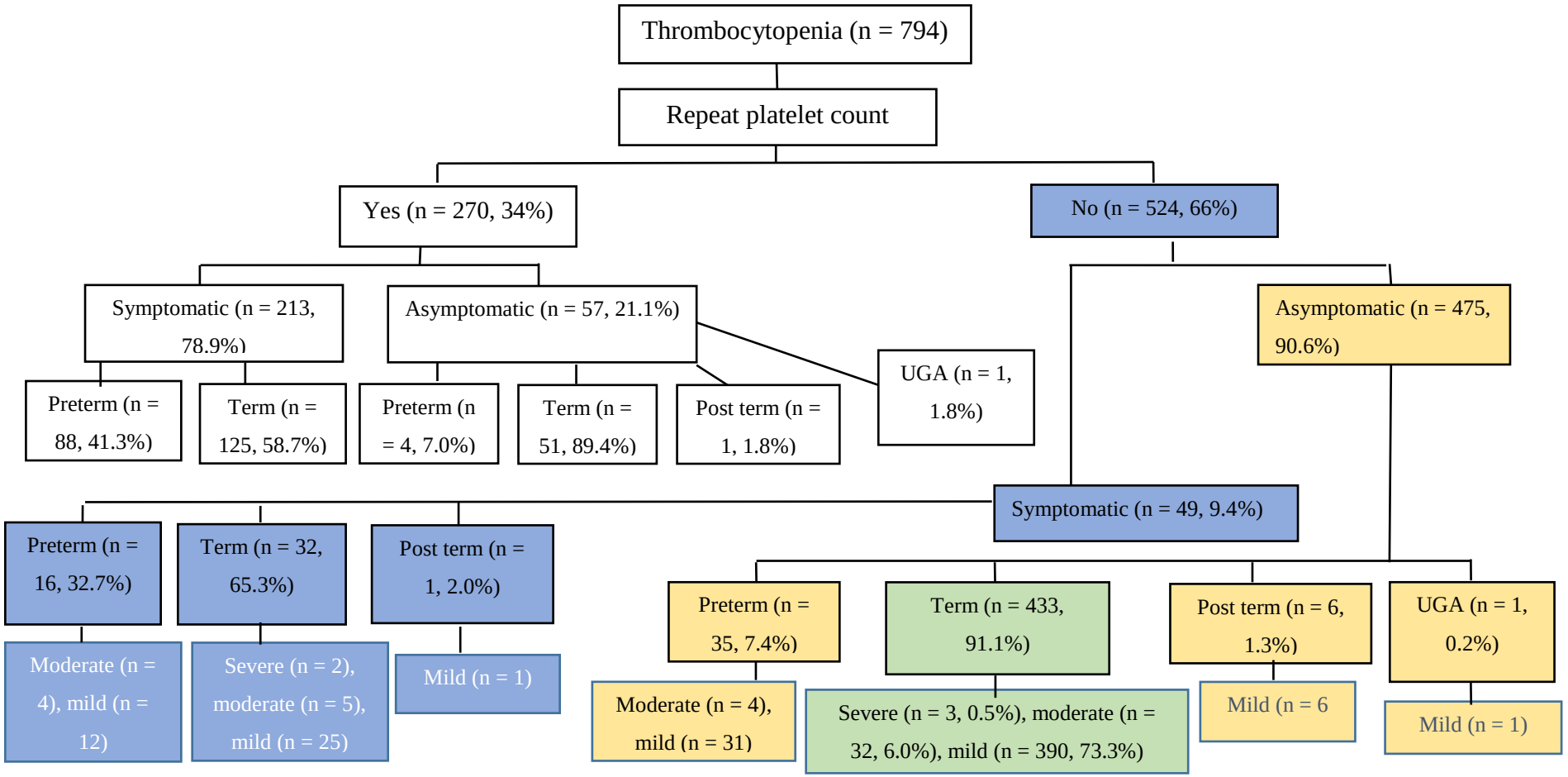

Figure 5.10: Repeat platelet counts in asymptomatic and symptomatic thrombocytopenic infants

5.8 Resolution of thrombocytopenia in symptomatic infants

Of the 213 symptomatic thrombocytopenic infants who had at least one repeat platelet count after birth, the thrombocytopenia resolved (i.e. recorded in the reference range) in 118 (55.4%) but resolution was not documented for the remaining 95 (44.6%).

Amongst those in whom the platelet count resolved, 6.6% were initially classified as having a severe thrombocytopenia, 7.0% as moderate and 41.8% as mild.

The results are shown in Table 5.18 and Figure 5.11.

5.8.1 Preterm Infants

The thrombocytopenia resolved in 63 (29.6%) symptomatic preterm infants who had repeat platelet counts. However, no resolution was recorded in the remaining 25 (11.7%). Amongst the 63 whose platelet count normalised, 1.9% were initially classified as severe, 5.6% as moderate and 22.1% as mild thrombocytopenia. Among the 25 (11.7%) infants who did not show resolution of the thrombocytopenia 0.9% were initially classified as severe, 2.8% as moderate and 8.0% as mild thrombocytopenia. Within this group, 16 infants remained thrombocytopenic, eight were reported as early neonatal death and in one infant the repeat platelet count was reported as clotted, so no result was available (Table 5.18 and Figure 5.11).

5.8.2 Term Infants

Amongst the 125 symptomatic term infants who were followed up, a repeat platelet count showed resolution of thrombocytopenia in 55 (25.8%) infants but not in the remaining 70 (32.9%). Amongst these babies 62 infants continued to demonstrate thrombocytopenia, three were reported as neonatal death, three had clumped platelets and two had clotted samples (Table 5.18 and Figure 5.11).

The number of days before documented resolution of the thrombocytopenia was recorded (Table 5.19).

Amongst the 14 infants with severe thrombocytopenia whose platelet count normalised, three had done so within one week, six within two weeks, four within three weeks and one took more than two months (Table 5.19).

Amongst the 15 infants with moderate thrombocytopenia whose platelet count normalised, two had done so in less than one day, six within one week, six within two weeks, and one took more than two months (Table 5.19).

Among the 89 infants with mild thrombocytopenia whose platelet count normalised, 28 infants did so on the same day, 39 within one week, 21 within two weeks, and one took more than two months (Table 5.19).

Thus, the time taken for recorded resolution of thrombocytopenia in symptomatic infants ranged from less than a day to more than two months (Table 5.19).

Table 5.18. Resolution of thrombocytopenia in symptomatic infants

	Repeat count resolved (n = 118, 55.4%)						Total resolved		Repeat count did not resolved (n = 95, 44.6%)						Total not resolved	
Gestation Age	**Severe**	**%**	**Moderate**	**%**	**Mild**	**%**	**Total**	**%**	**Severe**	**%**	**Moderate**	**%**	**Mild**	**%**	**Total**	**%**
Preterm	4	1.9	12	5.6	47	22.1	**63**	**29.6**	2	0.9	6	2.8	17	8.0	**25**	**11.7**
Term	10	4.7	3	1.4	42	19.7	**55**	**25.8**	1	0.5	20	9.4	49	23.0	**70**	**32.9**
Post Term	0	0.0	0	0.0	0	0.0	**0**	**0.0**	0	0.0	0	0.0	0	0.0	**0**	**0.0**
Unknown gestation age	0	0.0	0	0.0	0	0.0	**0**	**0.0**	0	0.0	0	0.0	0	0.0	**0**	**0.0**
Total	14	6.6	15	7.0	89	41.8	**118**	**100.0**	3	1.4	26	12.2	66	31.0	**95**	**100.0**

Table 5.19. Number of days to resolution of thrombocytopenia in symptomatic infants

Severity	**Days to resolve**														**Total**	
	< 1 day	**%**	**1 - 7 days**	**%**	**8 - 14 days**	**%**	**15 - 21 days**	**%**	**22 - 30 days**	**%**	**1 month**	**%**	**> 2 months**	**%**	**Total**	**%**
Severe	0	0.0	3	2.5	6	5.1	4	3.4	0	0	0	0	1	1.2	14	11.9
Moderate	2	1.7	6	5.1	6	5.1	0	0.0	0	0	0	0	1	1.2	15	12.7
Mild	28	23.7	39	33.1	21	17.8	0	0.0	0	0	0	0	1	1.2	89	75.4
Total	30	25.4	48	40.7	33	28.0	4	3.4	0	0	0	0	3	2.5	118	100.0

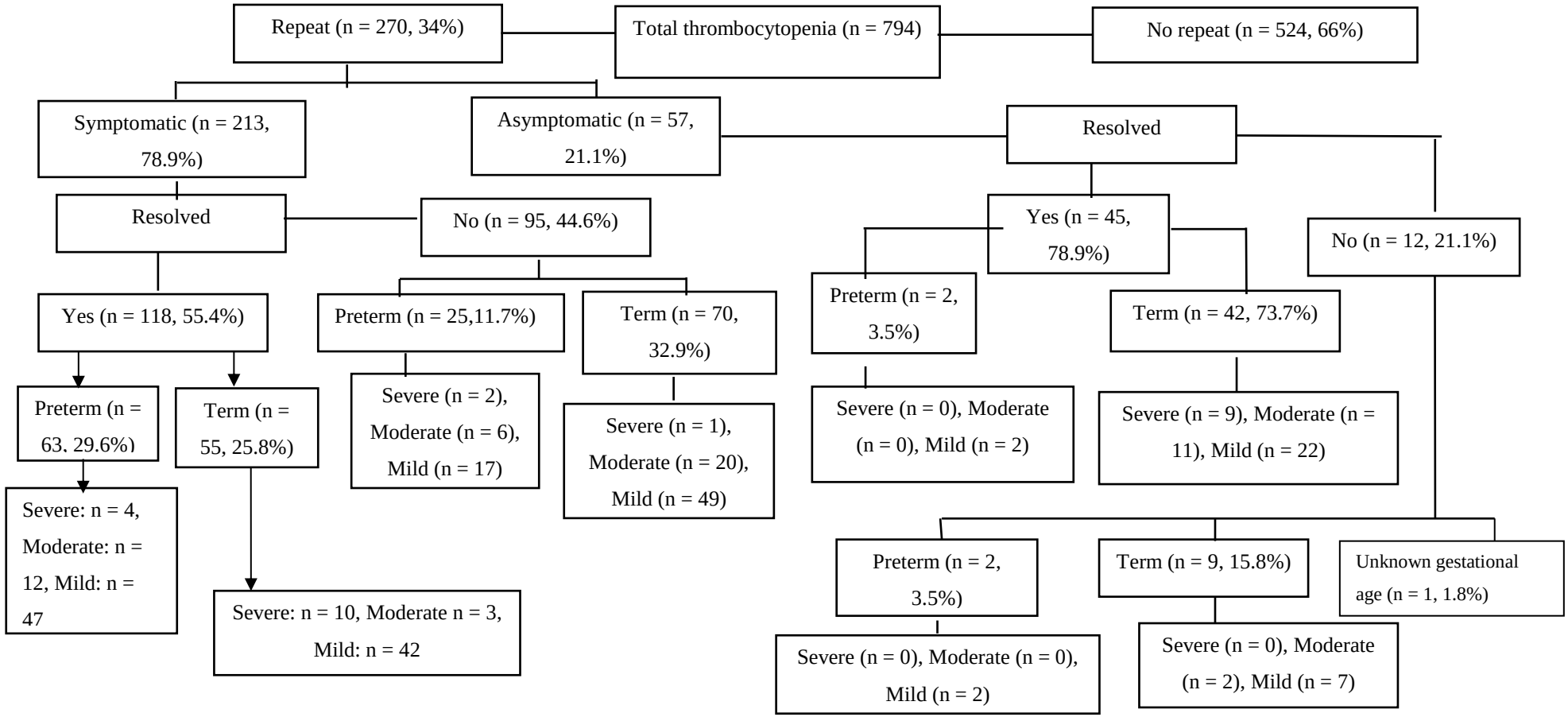

Figure 5.11: Resolution of platelet count in symptomatic and asymptomatic thrombocytopenic infants

5.9 Asymptomatic Infants

Only 57 (10.7%) of the 532 asymptomatic thrombocytopenic infants, had their platelet counts repeated. Thus 475 (89.3%) did not (Table 5.20 and Figure 5.10).

Amongst these 57 thrombocytopenic infants, 7.0% were preterm, 89.4% were term, 1.8% was born post- term, and 1.8% infant had no gestational age recorded.

Forty-five (78.9%) of the 57 infants demonstrated resolution of the thrombocytopenia and 21.1% did not.

5.9.1 Preterm Infants

Thirty-five (6.6%) asymptomatic preterm infants did not have a repeat platelet count. Four had moderate and thirty-one of these had a mild thrombocytopenia.

Of the four who did have a repeat platelet count, all were only mildly thrombocytopenic at birth (Table 5.20 and Figure 5.10).

5.9.2 Term Infants

Of the 484 asymptomatic term infants, 89.4% had repeat platelet counts and 91.2% did not (Table 5.20 and Figure 5.10).

Amongst the 51 who did have a repeat platelet count, there were nine of a possible 12 with severe thrombocytopenia, 13 of a possible 53 with moderate, and 29 of a possible 419 with mild.

Thus, amongst the 433 asymptomatic term infants who did not have a repeat platelet count there were three of a possible 12 with severe thrombocytopenia, 40 of 53 with moderate, and 390 of 419 with mild.

5.9.3 Post-term Infants

Only one infant out of the seven asymptomatic post term infants who were thrombocytopenic had a repeat platelet count. This baby initially had a moderate thrombocytopenia (Table 5.20 and Figure 5.10).

The remaining six infants who that did not have a repeat platelet count all initially had a mild thrombocytopenia.

5.9.4 Infants of Unknown Gestational age

Of the only two asymptomatic infants who have no record of gestational age, one had a repeat platelet count and the other did not. Both babies initially had a mild thrombocytopenia (Table 5.20 and Figure 5.10).

Table 5.20. Asymptomatic thrombocytopenic infants with (A) or without (B) repeat platelet counts.

A	**Repeat platelet count (n = 57, 10.7%)**													
Gestation age	**Severe (n = 9, 15.8%)**				**Moderate (n =14, 24.6%)**				**Mild (n = 34, 59.6%)**				**Total repeat**	
	Male	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**Total**	**%**
Preterm	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	4	**7.0**	0	**0.0**	**4**	**7.0**
Term	4	**7.0**	5	**8.8**	6	**10.5**	7	**12.3**	19	**33.3**	10	**17.5**	**51**	**89.4**
Post term	0	**0.0**	0	**0.0**	1	**1.8**	0	**0.0**	0	**0.0**	0	**0.0**	**1**	**1.8**
Unknown GA	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	1	**1.8**	**1**	**1.8**
Total	**4**	**7.0**	**5**	**8.8**	**7**	**12.3**	**7**	**12.3**	**23**	**40.3**	**11**	**19.3**	**57**	**100.0**

B	**No repeat count (n = 475, 89.3%)**													
Gestation age	**Severe (n = 3, 0.6%)**				**Moderate (n = 44, 9.3%)**				**Mild (n = 428, 90.1%)**				**Total no repeat**	
	Male	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**Male**	**%**	**Female**	**%**	**Total**	**%**
Preterm	0	**0.0**	0	**0.0**	2	**0.4**	2	**0.4**	16	**3.4**	15	**3.2**	**35**	**7.4**
Term	1	**0.2**	2	**0.4**	22	**4.7**	18	**3.8**	220	**46.3**	170	**35.8**	**433**	**91.2**
Post term	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	4	**0.8**	2	**0.4**	**6**	**1.3**
Unknown gestation age	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	1	**0.2**	**1**	**0.2**
Total	**1**	**0.2**	**2**	**0.4**	**24**	**5.1**	**20**	**4.2**	**240**	**50.5**	**188**	**39.6**	**475**	**100.0**

5.9.5 Resolution of thrombocytopenia in asymptomatic thrombocytopenic infants.

Amongst the 57 asymptomatic thrombocytopenic infants who were followed up, 79% demonstrated resolution of their thrombocytopenia, leaving 21% infants who did not.

Amongst the 45 (79%) infants, 15.8% initially had a severe thrombocytopenia, 21.1% moderate and 42.1% mild.

Amongst the 12 (21%) infants, none of the infants had a severe thrombocytopenia, 3.5% moderate and 17.5% mild.

The results are shown in Table 5.21 and Figure 5.11

Preterm Infants

Two of four asymptomatic preterm infants had repeat platelet counts. Two infants showed resolution, the repeat platelet count remained low in another, and in the remaining infant the repeat sample was clumped, precluding an accurate count (Table 5.21 and Figure 5.11).

Term Infants

In 42 of the 51 infants who were followed up, the thrombocytopenia resolved but not in the remaining nine infants. Of these, eight infants remained thrombocytopenic and in the remaining case the platelets were reported as clumped, thus precluding a result (Table 5.21 and Figure 5.11).

Post-term Infants

The thrombocytopenia resolved in the only post- term infant who was followed up (Table 5.21 and Figure 5.11).

Unknown Gestational Age

In the only infant of unknown gestational age, the thrombocytopenia did not resolve (Table 5.21 and Figure 5.11).

Amongst the nine infants with severe thrombocytopenia demonstrating resolution, this was achieved in four infants in less than a day, three within one week, one by two weeks, and one by three weeks (Table 5.22).

Amongst the 12 infants with moderate thrombocytopenia demonstrating resolution, this was achieved in two infants in less than a day, nine within one week, and two by two weeks (Table 5.22).

Amongst the 24 infants with mild thrombocytopenia demonstrating resolution, this was achieved in three infants in less than a day, 18 within one week, two by two weeks, and one took more than two months (Table 5.22).

Thus, the number of days after birth before the thrombocytopenia resolved for asymptomatic term infants ranged from two hours to more than two months (Table 5.22).

Table 5.21. Resolution of thrombocytopenia in asymptomatic infants.

	Repeat count resolved (n = 45, 79.0%)						Total resolved		Repeat count did not resolved (n = 12, 21.0%)						Total not resolved	
Gestation Age	**Severe**	**%**	**Moderate**	**%**	**Mild**	**%**	**Total**	**%**	**Severe**	**%**	**Moderate**	**%**	**Mild**	**%**	**Total**	**%**
Preterm	0	0	0	0.0	2	3.5	**2**	**3.5**	0	0	0	0.0	2	3.5	**2**	3.5
Term	9	15.8	11	19.3	22	38.6	**42**	**73.7**	0	0	2	3.5	7	12.3	**9**	15.8
Post Term	0	0	1	1.8	0	0.0	**1**	**1.8**	0	0	0	0.0	0	0.0	**0**	0.0
Unknown gestation age	0	0	0	0.0	0	0.0	**0**	**0.0**	0	0	0	0.0	1	1.8	**1**	1.8
Total	9	**15.8**	12	**21.1**	24	**42.1**	**45**	**100.0**	0	0	2	3.5	10	17.5	**12**	**100.0**

Table 5.22. Days to resolution of thrombocytopenia in asymptomatic infants

	Days to resolve															
	< 1 day	**%**	**1 - 7 days**	**%**	**8 - 14 days**	**%**	**15 - 21 days**	**%**	**22 - 30 days**	**%**	**1 month**	**%**	**> 2 months**	**%**	**Total**	**%**
Severe	4	8.9	3	6.7	1	2.2	1	2.2	0	0	0	0	0	0.0	**9**	20.0
Moderate	2	4.4	9	20.0	1	2.2	0	0.0	0	0	0	0	0	0.0	**12**	26.7
Mild	3	6.7	18	40.0	2	4.4	0	0.0	0	0	0	0	1	2.2	**24**	53.3
Total	9	**20.0**	30	**66.7**	4	**8.9**	1	**2.2**	0	0	0	0	1	**2.2**	**45**	**100.0**

5.10 Infants diagnosed with NAIT

Amongst the 10 cases of infants diagnosed clinically and/or serologically with NAIT, nine were followed-up and all demonstrated resolution of their thrombocytopenia. One baby (Number 242) had no record of resolution. The duration before documented resolution ranged from one day to more than two months (Table 5.23).

Table 5.23. Resolution of thrombocytopenia in infants with NAIT

Infant Number	**Cord blood platelet count (x 10^9/L)**	**Resolved platelet count (x 10^9/L)**	**Number of days taken to resolve**
1	5	183	2 weeks
2	6	203	1 day
4	7	216	2 weeks
14	27	152	1 day
17	32	213	2 months
18	32	163	10 days
26	41	152	2 months
45	59	211	2 months
209	110	269	1st repeat after 5 days and resolved after 2 months
242	115	(127)*	Not documented. Repeat platelet count after 9 days

Figure 5.12 shows the number of days after birth by which thrombocytopenia had resolved. It was assumed that an earlier platelet count remained less than the subsequent one, unless documented otherwise. (*Unresolved value)

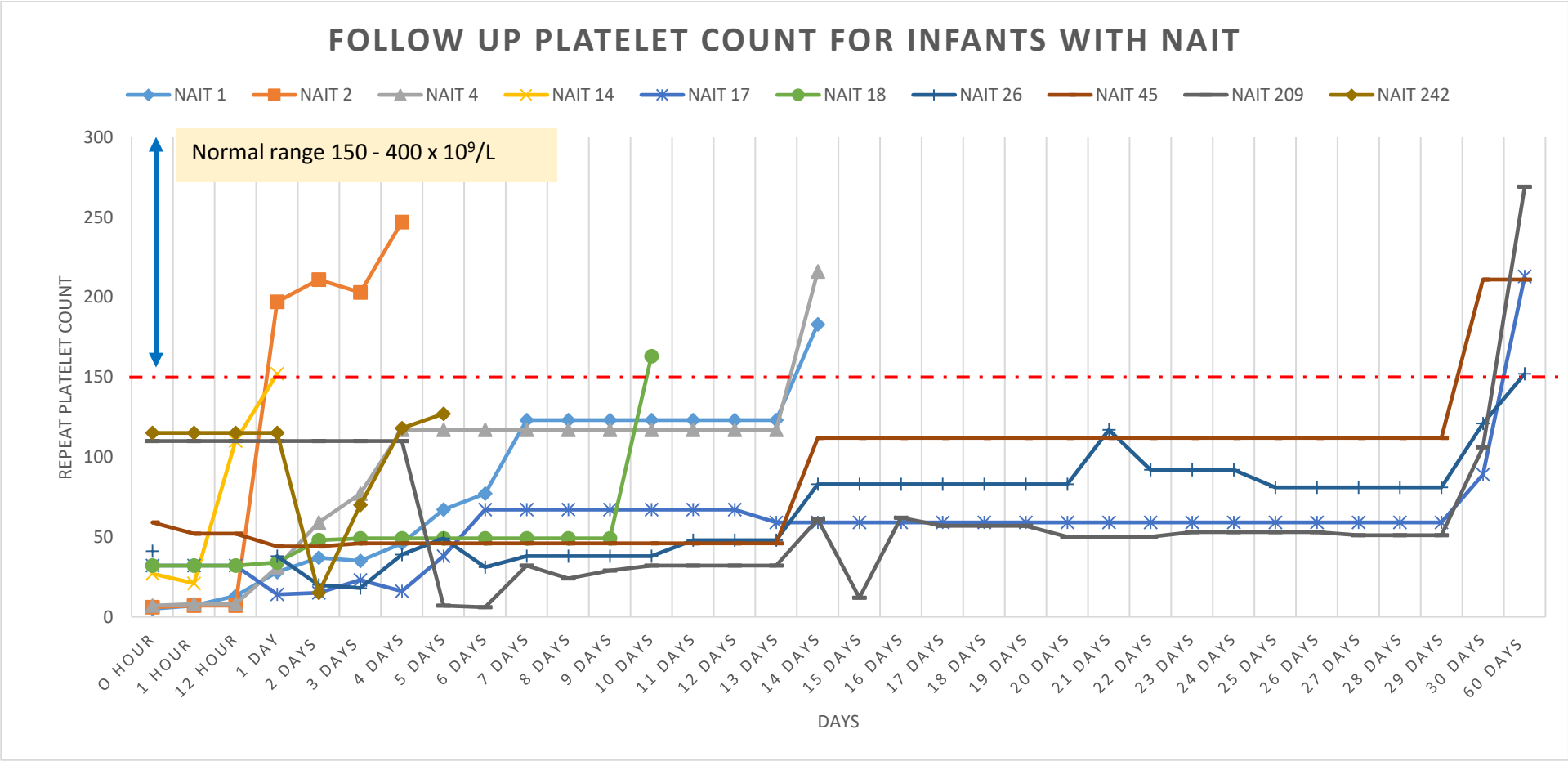

Figure 5.12. Time taken for thrombocytopenia to resolve in infants diagnosed with NAIT.

Chapter 6 Discussion

Thrombocytopenia is the most common disorder observed in newborn infants admitted to a neonatal intensive care unit (Carr et al., 2017). The causes of thrombocytopenia vary according to the underlying disease and can be classified according to the time at onset (Fustolo-Gunnink et al., 2016). Although it may be the most common haematological disorder it is however not the commonest otherwise. Neonatal thrombocytopenia has numerous different aetiologies, and can be congenital or acquired (Peters & Grainger, 2017). Among the causes, NAIT is identified as the most common cause of severe thrombocytopenia (about 27%) in term infants, and accounts for 3% of all cases of neonatal thrombocytopenia (Tiller, Husebekk, Ahlen, Stuge, & Skogen, 2017).

The objective of this study was to determine the prevalence and clinical outcomes of thrombocytopenia in healthy term infants born at Middlemore Hospital, Auckland, New Zealand over an eleven – year study period. However, it was not possible to determine the incidence from the beginning considering the definition of incidence therefore prevalence was used instead.

In this study, all cord blood results were audited, and platelet counts less than 150 x 10^9/L were selected and used for the analysis. The first aim of this research was to determine the prevalence of thrombocytopenia in otherwise healthy term infants of varying ethnicities. However, the incidence of thrombocytopenia cannot be determined as there was no prospective study done and this was a retrospective study. However, it would be better to do prospective to determine the true incidence of thrombocytopenia.

Incidence is determined by the probability of new cases of a given medical condition in a given population at risk within a given period of time (Bhat, Naik, Rafiq, & Tariq, 2015; Gupta et al., 2011).

On the other hand, prevalence was calculated using the data from the audit. Several studies have reported that the incidence of neonatal thrombocytopenia is between 0.5 – 0.9% and the prevalence differs amongst different populations studied (Roberts et al., 2008; Ulusoy et al., 2013; Yougbare, Zdravic, & Ni, 2018).

This study found that the prevalence of neonatal thrombocytopenia amongst babies born at Middlemore Hospital is 1.2%, with a slight male predominance. The finding of this study is consistent with other studies which report the prevalence of thrombocytopenia in newborn populations as 1% to 5% (Carr et al., 2017; Sainio et al., 2017; Sanii, Khalessi, Khosravi, & Zareh Mehrjerdi, 2013).

In this study, the majority of the thrombocytopenic cases were mild (82.4%), followed by moderate (13.7%) and severe (3.9%). These findings are similar to other studies which report that mild and moderate thrombocytopenia comprise the majority (and up to 75%) of cases of neonatal thrombocytopenia, whilst the prevalence of severe thrombocytopenia ranges between 2.5 and 25% (Gupta et al., 2011; Roberts et al., 2008; Ulusoy et al., 2013). The majority of cases of mild and moderate thrombocytopenia do not require intervention (Gunnink et al., 2014; Roberts et al., 2008), since most are self-limiting and tend to resolve within a short time (Chakravorty & Roberts, 2012; Roganovic, 2015). This agrees with the findings of this study. Moreover, studies have shown that severe thrombocytopenia is uncommon, occurring in 0.1% to 0.5% of the general newborn population (Penel-Page et al., 2017; Roberts et al., 2008), and 5% to 22% of neonates in neonate intensive care units (Tiller et al., 2017).

Although thrombocytopenia is prevalent it is often overlooked, presumably because t is expected that it will resolve spontaneously. It is babies with severe thrombocytopenia who require intervention, as if they are not treated the thrombocytopenia can result in devastating consequences such as ICH (Gupta et al., 2011).

The findings of this study show that in about 35.5% of cases of severe thrombocytopenia in term infants there is no documented cause, and another 22.6% are thought to be due to NAIT. These findings are similar to other studies documenting that, among severely thrombocytopenic newborn infants, NAIT is diagnosed in 25% (Meler et al., 2017). The high number of cases with severe, unexplained thrombocytopenia are cause for concern as this could have a devastating effect on the infants since most common cause of severe thrombocytopenia in term infants is NAIT. Some studies have reported that the cause of severe thrombocytopenia is often not identified (Carr et al., 2017). Possible explanations for these unexplained cases of thrombocytopenia have been suggested and include multiple aetiologies such as a defect in megakaryopoiesis affecting the regulation of platelet production, impairment of megakaryopoiesis due to placental insufficiency, systemic inflammation related to perinatal infection or

chorioamnionitis without infection and the method of blood collection which could affect the platelet results (Carr et al., 2017; Venkatesh, Curley, & Stanworth, 2015). Furthermore, studies have reported that the unexplained thrombocytopenia could be due to the presence of a number of rare disorders or inherited thrombocytopenia which present with thrombocytopenia at birth (Roberts et al., 2008). Although most cases of severe thrombocytopenia have unexplained causation, several authors recommend that infants should always be assessed and investigated for a cause of thrombocytopenia, taking into consideration the medical and family history, bleeding symptoms such as petechial rash and incidental thrombocytopenia finding (Peters & Grainger, 2017). Similarly, it is recommended that any term infant with severe thrombocytopenia should be investigated to establish a cause (Venkatesh et al., 2015).

In the current study of babies born at Middlemore Hospital, no attempt at explanation for unexplained cases of severe thrombocytopenia is documented; the records simply state "thrombocytopenia". This highlights the importance of proper record keeping and follow up of thrombocytopenia by checking on the baby and repeating the platelet count. It could be assumed that the lack of documentation around thrombocytopenia reflects incomplete record- keeping in the hospital databases. However, this seems unlikely as the laboratory results would have been documented if a repeat platelet count had been performed. Other reason could include the possibility that an infant's information is not recorded because it was born at another birthing facility and/or that its family has moved out of the area. Every thrombocytopenic sample should be checked, and if necessary repeated, to exclude pseudo-thrombocytopenia due to clots or platelet clumping when the blood sample is collected in EDTA containing tubes. Clotted or clumped samples should be repeated and repeat collection for clumped samples should be in a different anticoagulant such as sodium citrate. If the repeat sample still shows thrombocytopenia, then causes of the thrombocytopenia should be investigated (Gauer & Braun, 2012).

In addition, well term babies with low counts would likely not have a diagnosis of the cause. Does this matter in practice? To answer this question the subsequent clinical records of the infants and their families were audited to determine if there was any evidence of NAIT being missed. However, there was no evidence that NAIT was missed. The clinicians usually have follow up done since most healthy term would have a presumed caused. On the contrary, if there is no evidence of the cause of thrombocytopenia then there is no need for screening and follow up.

The cord blood samples were requested by Haematologist as this was a classic case of a routine test i.e. cord count and not requested by a clinician. Therefore, if the ward is unaware then nobody followed up on it since they did not request it. If the laboratory detects any abnormality should report to clinicians or wards responsible.

This study found that term infants were the commonest group with thrombocytopenia, accounting for more than four-fifths of babies. Most of the remainder were preterm infants, with only a few post term. The ratios of term (641): preterm (143): post term (8). Using a combination of hospital data- bases it was possible to record the gestational age on almost all infants. The predominance of term infants could reflect the fact that there are more term infants born than preterm infants and the thrombocytopenia is proportional. The preterm birth rate is about 7% of the total. So, the fact that 18% of the thrombocytopenic ones were preterm indicates this problem is commoner in the preterm.

Moreover, it is not common practice in most centres to check the full blood count on healthy term infants, so the prevalence of thrombocytopenia in this group may not be truly known. However, other studies do report that preterm infants are more likely to develop thrombocytopenia than any other gestational age group (Carr et al., 2017; Chakravorty & Roberts, 2012; Gunnink et al., 2014; Roganovic, 2015). Moreover, it has been documented that infants born at term normally show normal platelet counts (Carr et al., 2017). A possible explanation of this may be that a lower reference range for the platelet count in preterm infants was used, such as that established by Wiedmeier, Henry, Sola-Visner, and Christensen (2009), or that platelet count at birth differs between cord (as in this study) and peripheral blood, as analysed in Wiedmeier's report. Wiedmeier et al. (2009) found that in infants born between 22 and 32 weeks the lower limit of the platelet count is 104 x 10^9/L (5^{th} centile), and in infants born after 32 weeks (late preterm) the lower limit of the platelet count is 123 x 10^9/L. It is suggested that a platelet count between 100 and 150 x 10^9/L is considered normal in most preterm infants. In using this reference ranges, there will be fewer cases documented as thrombocytopenia, and needing to be investigated (Wiedmeier et al., 2009). At Middlemore Hospital, neonatal staff adhere to the adult reference range for platelets. However, thrombocytopenia in preterm is usually due to pathological consequences which influence the results of the full blood count (Carr et al., 2017).

This study uses the cord blood reference range (150 – 400 x 10^9/L) established by Middlemore Hospital Haematology Laboratory, therefore infants whose platelet count is below this reference range are regarded as thrombocytopenia.

Platelets play significant roles in thrombosis, inflammation and wound repair, and an abnormal platelet count is an indication of a disease and/or disorder. Therefore, it is important to perform a FBC which is useful in neonatal medicine (Wiedmeier et al., 2009).

Neonatal thrombocytopenia is defined as a platelet count of less 150 x 10^9/L. Each testing laboratory should review its own reference ranges (Katsares et al., 2009), as this aids in identifying cases of thrombocytopenia as well as classifying degrees of severity. Regardless of gestational age, the same reference ranges are used, although this is controversial, as with preterm infants (Cremer et al., 2016; Wiedmeier et al., 2009). Moreover, there is no clear- cut evidence regarding the applicability of adult reference ranges to the newborn, and some studies indicate that healthy preterm and term infants can have platelets below the adult reference range (Cremer et al., 2016).

This study shows that in most cases, the cause of thrombocytopenia was not recorded. Moreover, as only about one third of the infants were admitted, it appears no attempt at explaining the thrombocytopenia was attempted in the remainder. The possible reasons are that most cord blood samples were requested by the Haematologist and there is no correlation as to who is responsible for the follow up.

In the majority of cases, the cause of thrombocytopenia amongst infants admitted to hospital was not identified. The infants admitted refers to those infants that are admitted in NICU with identified causes for thrombocytopenia as reasons for admissions, primary and secondary diagnosis.

A known causes or disorder likely to cause thrombocytopenia was only identified in about one fifth of the infants. NAIT accounts for 1.3% of the causes of thrombocytopenia for the thrombocytopenic infants that were admitted.

The findings of this study show that conditions most likely to cause thrombocytopenia other than NAIT in infants admitted based on primary diagnosis are, in order of decreasing magnitude, prematurity (48.1%), RDS (20%), hypoglycaemia (11.3%),

IUGR (6.3%), Downs syndrome (5%), sepsis (3.1%), perinatal asphyxia (3.1%), and thrombocytopenia (1.3%)

These findings are similar to other studies which report that, in the vast majority of cases, thrombocytopenia in newborns is a consequence of other significant co-morbidities such as prematurity, infection, IUGR, sepsis, and LBW (Carr et al., 2017; Gupta et al., 2011). Less common causes of thrombocytopenia include neonatal alloimmune thrombocytopenia (NAIT), thrombocytopenia due to maternal autoimmune thrombocytopenia (ITP) and the hereditary thrombocytopenias (Carr et al., 2017).

The findings of this study show that over two thirds of the thrombocytopenic infants are asymptomatic infants, mostly born at term; over half of them had a mild thrombocytopenia.

These findings are similar to several other studies which report that, in the absence of a screening program, the majority of infants recorded are asymptomatic and only a few cases are symptomatic (Madani, Kamphuis, Lopriore, Porcelijn, & Oepkes, 2012). However, even a moderate thrombocytopenia may still be asymptomatic (Gauer & Braun, 2012).

The second aim of the study was to investigate associations of thrombocytopenic infants with clinical outcomes in affected infants and siblings.

For this study, data collection covers a 11 - year period, during which there were a total of 68910 births with an average of 6000 births per year. Middlemore Hospital is the major hospital for the Counties Manukau district. It offers secondary and tertiary level (hospital and specialist) care and a selected range of community and domiciliary services for the population of Counties Manukau. It is one of the largest tertiary teaching hospitals in New Zealand. According to Counties Manukau webpage (www.countiesmanukau.health.nz, n.d), the district has a high birth rate compared with many other areas. This is one of the contributing factors which puts high demand on the hospital's maternity, child and youth health services. According to the annual report for women's health, and the newborn annual report 2016 to 2017, the mortality and hospitalisation rates are higher than the national rates, with Pacific residents (30.6%) and Maori (22.9%) exhibiting higher rates than those in Asian (22.6%) and NZ European/Other groups (23.8%) (www.countiesmanukau.health.nz, n.d).

The ethnicities for mothers with asymptomatic infants identified in this study are 38.8 are Polynesians, 20.3% are Europeans, 20.1% are Asians, 16.7% are Maori and 2.1% are others. CMDHB report 2016 shows relative percentages of births by ethnicity. It might be expected that if there was no ethnic bias to thrombocytopenia the proportion of each ethnic group in the thrombocytopenia infants would be similar. However, it appears that there is a slightly greater preponderance of Pacific Islands (40.4%) than the expected value of about 28%.

Although there is no screening program in New Zealand, Auckland District Health Board (ADBH) has provided information on the possible alloimmunisation of HPA during pregnancy (www.adhb.govt.nz, n.d). It is reported that Europeans are incompatible with HPA – 1a, HPA – 5b and – 15b, Maoris are incompatible with HPA – 2b and HPA – 3a, Asians are incompatible with HPA – 4b, and Polynesians are incompatible with HPA – 6b (www.adhb.govt.nz, n.d). Currently the Tissue Typing Laboratory at New Zealand Blood Service (NZBS) is testing for NAIT using the following the methods; IMMUCOR PAK - LM™ (Luminex Assay) which detects serum platelet antibody; linkage Biosciences LinkSēq™ HPA genotyping kit, and flow cytometry platelet immunofluoresence test (FC – PIFT). The antigens tested include HPA – 1, - 2, -3, -4, -5 and -15. The other parameters tested include HLA class 1 and glycoproteins. The possibility of missing these anti - HPA antibodies could result in serious medical implications. If HPA antibodies are not detected demonstrates that there are no HPA genotype incompatibilities and thrombocytopenia is probably due to HLA or blood group antibodies.

As New Zealand is a heterogenous multicultural country with lot of people migrating to the country and intermarriages it will be difficult to select suitable donor for transfusion (Edinur et al., 2013; www.adhb.govt.nz, n.d). Edinur et al. (2013) state that identifying the HPA frequency in different ethnicities is important because it may have medical implications and inform the risk of platelet alloimmunisation.

However, not all allele frequencies are being studied for different ethnicities and in some ethnicities the platelet specificity is unknown. Meanwhile, there are rare HPA that are not known and tested (Edinur et al., 2013; Lin et al., 2018).

The advantages of knowing the frequencies of HPA in different ethnicities, may be of diagnostic value and relevant to platelet (Shaiegan et al., 2011).

Testing for HPA antibodies is complicated by the fact that HPA genes and variants, other than the ones tested, will be missed. In addition, contamination of the cord blood by maternal cells can give a false result and rare HPA genes can give diagnostic errors (Gérald Bertrand & Kaplan, 2017; Crighton et al., 2017; Hopkins et al., 2017).

The study found that 1.9% of the mothers who had asymptomatic infants had stillbirths, and 20.5% of the women had experienced miscarriages either prior to or after the recorded birth. These results are consistent with Reiher et al. (2017) who found 21% of the women had prior miscarriages. Although stillbirths and miscarriages are recorded in women who had asymptomatic infants, there are no documented cases of NAIT in these women. On the other hand, this study found that about 57.1% of the cases did not have post mortem therefore the causes were not identified. This finding is similar to several studies that documents that in most cases, it is difficult to identify the aetiology, therefore the cause of the stillbirth cannot be established without further assessment. However, if the stillbirth is examined clinically and if pale from haemorrhage this is regarded as abnormal (Silver et al., 2007).

Since there is no screening program for NAIT, most records of the mothers were normal and there are no records of any association of these women with NAIT. Thus, it can be assumed that the stillbirths and miscarriages are probably not caused by NAIT.

Several studies have documented that any woman with a foetal loss as a result of thrombocytopenia should be tested for HPA alloimmunisation in subsequent pregnancies. The serology tests are performed to detect HPA and the parents should be tested. If the HPA antibody is not detected, the parents should be tested to see if there is any possibility of HPA incompatibility (Vadasz et al., 2015).

Most causes of stillbirths are unknown since most of the post mortems were declined. Thus, intrauterine death associated with NAIT may have been under- estimated. This concurs with other studies which have shown that the number of cases of stillbirth associated with NAIT are unknown since infants do not usually have an autopsy (Kjeldsen-Kragh et al., 2007).

Thus, most studies report that the number of NAIT cases due to stillbirths are yet to be established, even though there may be mention that the mother had prior stillbirths (Kjeldsen-Kragh et al., 2007).

This study reports that 20.5% of the mothers had miscarriage prior to birth. Since there is no screening program none were tested for NAIT.

Studies have demonstrated that a maternal immune response against foetal HPA alloimmunisation in NAIT progresses to clinical complications including bleeding disorders ranging from mild cutaneous petechiae to severe intracranial haemorrhages (ICH), intrauterine growth restriction (IUGR), and foetal or neonatal death (Vadasz et al., 2015; Yougbare et al., 2018). On the other hand, this study identifies infants born to pre-eclamptic mothers which was also observed in other studies as well (Gupta et al., 2011).

According to Tiller et al. (2017), mothers who give birth to infants diagnosed with NAIT often report a miscarriage as part of their clinical detail and history. In addition, it is documented that the risk of miscarriage may be increased.

Furthermore, it was found using murine model of FNAIT, that maternal anti – integrin β3 antibodies implicate foetal miscarriage. It is demonstrated that anti – integrin β3 antibodies activate placental natural killer cells which damage the placenta causing ensuing miscarriage through antibody dependent cellular cytotoxicity (Tiller et al., 2017).

Although miscarriages are reported as devastating outcomes in NAIT, there is a lack of understanding of the pathogenesis of the disease and treatment options. In addition, the incidence and mechanisms of miscarriages are often paid less attention, thus it is a topic for further study (Tiller et al., 2017; Vadasz et al., 2015; Yougbare et al., 2018; Zdravic et al., 2016). However, it can be difficult to determine the rates of miscarriages as this can include termination of pregnancies.

The study found that 96.1% of the women had normal platelet count, 2.6% had low platelet count at birth, and 1.3% of the women do not have record either on Concerto or Healthware databases. These finding are similar to those documented by Roberts et al. (2008) where more than 98% of the neonates had mothers who had a normal platelet count at the time of their baby's birth. However, Yan, Malinowski, and Shehata (2016) report that 7 – 12% of pregnant women have thrombocytopenia during second or third trimester.

The study found that about 50% of the mothers who had thrombocytopenia had ITP, which comprised 1.3% of the total mothers with asymptomatic infants. It is widely reported that ITP accounts for about 3% of all cases of thrombocytopenia in pregnancy. Most pregnant women present with a history of ITP prior to or after birth (Ciobanu, Colibaba, Cimpoca, Peltecu, & Panaitescu, 2016), whilst thrombocytopenia during pregnancy is a common occurrence which can be due either to physiological changes and/or a pathological cause (Perepu & Rosenstein, 2013).

The audit records 532 asymptomatic thrombocytopenic infants over the 11 – year study period from January 2005 to June 2016. These comprise 60% of all thrombocytopenic babies during this time. The majority of the infants were term infants. Since almost half of the thrombocytopenic cases were asymptomatic term infants, there is a real chance that asymptomatic cases of NAIT were not diagnosed. Most of these infants survived without any problems and were discharged from follow-up after their platelet counts resolved. Since there is no screening program for NAIT in New Zealand, it is assumed that some cases may have been missed. Keeping a proper record of affected infants and their families is relevant because future pregnancies for the mother are at risk. Thus, she can be monitored and offered relevant medical interventions should the need arise. Without proper record keeping, future siblings may unnecessarily suffer the consequences of NAIT.

Studies have demonstrated that the prevalence of ICH in NAIT is small but significant. Thus, it is important that all suspected cases of NAIT and infants with a maternal history of NAIT, are followed up. Since the allele and genotype frequencies of HPA vary among different ethnicities, these should all be screened for possible NAIT (Eyada et al., 2018; Holzhauer & Zieger, 2011).

The frequency of HPA differs among ethnic groups across the globe. Thus more than 70% of Caucasians are positive for HPA – 1a, HPA – 5b, and HPA – 15b. Amongst Maori, most are positive for HPA – 2b and HPA – 3a, Asians are positive for HPA – 4b, and Polynesians are positive with HPA – 6b (www.adhb.govt.nz, n.d). NAIT testing is performed at the Tissue Typing laboratory at NZBS and if ethnically relevant HPA are not tested, there is the possibility that platelet specific antibodies could be missed. Some authors advise caution when analysing certain HPA such as HPA – 3 and – 15, because it is understood that the alleles are heterogeneous in the population (Ouabdelmoumene et al., 2018).

With variations in frequency of HPA amongst different ethnic groups, it would be helpful to study the occurrence of allo-immunisation by ethnic-specific HPA and the clinical consequences in different populations. It is essential that this information is relevant for the correct diagnosis of NAIT and specific treatment (Ouabdelmoumene et al., 2018). With frequent migration and intermarriage in New Zealand, such information should be available at testing centres because of its medical implications and the potential for antigenic mismatch which could result in platelet-specific allo-immunisation (Edinur et al., 2013). Thus the identification of allele frequencies of HPA in different ethnicities has implications not just for NAIT but also for clinical transfusion practice (Eyada et al., 2018). It is recognised that alloimmunisation against HPA contributes significantly to clinical complications in different ethnicities and with varying severity (Ouabdelmoumene et al., 2018).

The incidence of ICH due to NAIT in asymptomatic infants can only be determined by a radiological analysis. With the assumption that ICH cases due to NAIT were missed in asymptomatic infants could possibly have medical implications is suggestive of a future screening program for NAIT. A screening program is advantageous in that it provides information on the burden of the disease, incidence and prevalence, and the molecular methods which are already functional at the Tissue Typing Laboratory at NZBS can be validated and expanded to reflect the population being tested.

Some asymptomatic infant's siblings do not have records of thrombocytopenia because they were born before the databases were introduced or they were born in different districts, birthing units or overseas.

Since some of the asymptomatic infants (29.1%) do not have siblings therefore the true aetiology cannot be determined and some do not have record in the databases. Inconsistencies in record keeping can have serious medical consequences since NAIT can resolve spontaneously. Whilst the affected infant is now no longer vulnerable, the relevance of keeping a proper record is to safeguard future pregnancies and offspring.

Auckland District Health Board (ADHB) has an established protocol for the treatment and follow up of cases of NAIT (www.adhb.govt.nz, n.d). It is recommended that CMDHB adopt this protocol or develop their own or implement it if already established.

The third aim of this research was to develop a suggested guideline for the future detection and management of NAIT in New Zealand.

Only about one third of thrombocytopenic infants were recalled for a repeat platelet count. Thus, two thirds were not. Most of the infants having repeat platelet counts were symptomatic infants. Most asymptomatic infants were not followed up for a repeat platelet count. Ideally all infants should be followed up until the platelet count resolves. However, this study failed to document any adverse consequences to an infant remaining thrombocytopenic for a period after birth. This was true both amongst those infants who did have repeat platelet counts and, presumably, those who did not, as a repeat admission to Middlemore Hospital would have been detected during data collection had this been the case.

Several authors recommend that the platelet count be repeated between days 1 to 5 as it has been observed to be at its lowest during this time then rise spontaneously by day 7 (Brojer et al., 2016; Jovandric, 2015; Roberts et al., 2008).

The rationale for follow up is to monitor the platelet count until safer levels are achieved. In cases with severe thrombocytopenia, follow up should be maintained at least until the platelet count exceeds about 50 x 10^9/L to prevent the infant from the risk of ICH (Refsum, Hakansson, Mortberg, Wikman, & Westgren, 2018). Treatment is recommended if ICH is detected, but otherwise no treatment is required (G. Bertrand & Kaplan, 2014).

Studies have demonstrated that the platelet count should be observed and evaluated for at least a week to identify any abnormal results in the platelet count (Brojer et al., 2016; Risson et al., 2012).

This study found that one of the cases diagnosed with NAIT (number 209) had a mild thrombocytopenia at birth but was discharged. A repeat platelet count was only requested after the baby was readmitted on Day 5 with a petechial rash. The repeat count showed a severe thrombocytopenia and was followed up until it finally resolved two months later. This case indicates how significant follow up can be. In such cases it is recommended that the platelet count be repeated after noticing thrombocytopenia and followed up for at least a week until the count reaches a safer level (Brojer et al., 2016). If, however, as is currently the case at Middlemore Hospital now that the practice of performing routine cord blood FBC's has been discontinued, no FBC was performed at birth, this would have gone unnoticed until the baby presented with the rash.

Resolution of platelet count in babies with NAIT resolved between one day and up to 2 months after birth. These results are similar to those obtained by Jovandric (2015) and Reiher et al. (2017) who report that most cases of neonatal thrombocytopenic usually begins to resolve 7 to 10 days after birth, but may take up to 4 – 6 weeks.

On the other hand, most asymptomatic thrombocytopenic infants were never followed up, therefore without a screening it can be assume that some mild cases of NAIT may have been missed. Brojer et al. (2016) suggest that every year many NAIT cases are not detected and thus they avoid proper diagnosis and treatment.

In most cases, the first repeat platelet count was requested very soon after the initial result was issued. Since cord bloods used to be a routine request, doctors and midwives may not always have been alerted to a low platelet count, hence no request for follow up. In cases like this, and where HPA alloimmunisation might occur a few days after delivery (Reiher et al., 2017), babies with NAIT would have been missed. Moreover, studies have demonstrated that maternal HPA antibodies, usually IgG, can persist for approximately 3 months in the foetal circulation. Thus thrombocytopenia can persist as it depends on the rate of removal of maternal anti - HPA antibodies from the neonatal circulation (Abraham et al., 2018). It is, therefore, essential to plan to follow the disease for longer duration until the platelet count has normalised (Reiher et al., 2017).

Babies with severe thrombocytopenia frequently require platelet transfusions and should continue to be monitored in order to maintain a safe platelet count above which bleeding is unlikely. Thrombocytopenia can persist for several weeks to months (G. Bertrand & Kaplan, 2014; Roberts et al., 2008). According to Auckland District Health Board Newborn Services Clinical guidelines (www.adhb.govt.nz (n.d), affected infants should have a repeat platelet count daily until the count reaches a stable level over $100x10^9$/L.

Although a cord blood sample may be more likely to clot, how a platelet count can resolve within a short time frame such as within 1 day in otherwise healthy newborns is uncertain. Several studies have demonstrated that platelet count can remit spontaneously no later than the following day not only in healthy infants, but in infants who have medical conditions other than thrombocytopenia (Sainio et al., 2017).

It is however reported that although thrombocytopenia is prevalent, in most cases it is often ignored due to an assumption that it will resolve (Gupta et al., 2011). Studies have shown that most cases of moderate and mild thrombocytopenia resolve without

intervention (Sola-Visner et al., 2008). Most neonatal thrombocytopenia cases do not show symptoms and the platelet count spontaneously resolves within 1 week after birth without any treatment (G. Bertrand et al., 2017).

According to Auckland District Health Board Newborn Services Clinical Guidelines, if an infant's platelet count fails to respond to platelet transfusion, an alternative diagnosis should be considered or an attempt made to investigate for rarer maternal antibody types such as HLA (www.adhb.govt.nz, n.d).

The study findings show that most asymptomatic thrombocytopenic infants were never followed up or do not have a record of a repeat platelet count. Most of these infants had moderate or mild thrombocytopenia and do not require further intervention. The findings are consistent with several studies that moderate mild thrombocytopenia do not warrant interventions (Brojer et al., 2016; Kotwal, 2011).

The fourth aim of the research was to identify cases of NAIT in asymptomatic infants.

In the eleven – year study period, ten cases of NAIT were identified from 794 thrombocytopenic infants. The incidence of NAIT was not determined since this is a retrospective study. The estimated prevalence was calculated to be 1.3% (10/794). Thus, it is estimated that approximately 1 newborn per 80 births may be affected by NAIT. The findings of the study show that NAIT occurs in 22.6% of term infants with severe thrombocytopenia and comprise 1.6 % (10/641) of total term infants. However, only three cases were serologically confirmed cases of NAIT with HPA – 1a; the remaining cases were not tested or confirmed.

The majority (70%) of the cases had severe thrombocytopenia, 10% (1/10) moderate, and 20% (2/10) mild.

The estimated prevalence of NAIT, at 1.3% in this study is low compared to published data, where the reported prevalence ranges from 2.1% to 10.6% (Brojer et al., 2016). This wide range probably reflects differences in size and possibly ethnicity of study populations. This is a retrospective study based on an audit; however, a prospective study is recommended at Middlemore Hospital so that the true prevalence and even incidence may be known. In addition, several studies have suggested that knowledge of prevalence will aid in understanding the potential for NAIT in infants with platelet – related disorders, and also potentially encourage the blood service to compile a list of

compatible donors. This will then improve the safety and efficacy of platelet transfusion (Al-Ouda et al., 2016). However, with large data in this study, there is no evidence to justify that other health priorities would be discernible benefits.

Only three cases were confirmed as NAIT in which two were siblings (number 1 and 4) who also had two siblings with severe thrombocytopenia. The other confirmed case (number 2) had no record of siblings. Many studies have demonstrated that the recurrence rate of NAIT in subsequent pregnancies is reported to be high as 100% (Salomon & Rosenberg, 2013). The rate of recurrence cannot be determined in this study as most cases do not have siblings and two with siblings have no record of their haematology results. There should be records of all NAIT cases whether it was confirmed or suspected so that the subsequent pregnancies are monitored to reduce the risk of severe bleeding such as ICH.

Three confirmed infants (number 1, 2 and 4) with NAIT had mothers who were Europeans and confirmed as having HPA – 1a antibodies. These results are similar to other studies where almost 80% of the Caucasian population is HPA – 1a positive, and less than 2% are HPA – 1a negative (Lin et al., 2018). The seven cases that were not tested include two cases who were Europeans, three were Polynesians and two were Asians. According to ADBH newborn clinic services, the most common HPA involved in alloimmunisation among Europeans are HPA – 1a, - 5b and -15b, New Zealand Maori, HPA - 2b and HPA – 3a, Asians, HPA – 4b, and Polynesian, HPA – 6b (www.adhb.govt.nz, n.d). Without a screening program, there is an increase risk in these populations of not detecting and/or preventing foeto-maternal or post-transfusion alloimmunization (Brouk, 2015). In addition, diagnosis of NAIT is confirmed by detection of anti HPA – 1a antibodies in a mother who is HPA – 1a negative. However, in some cases diagnosis is not made on time which can result in the occurrence of ICH. This study did not find any obvious ICH in term infants, however, missing the diagnosis could have potentially dangerous consequences for affected neonates. The findings of the study show that the majority of infants with a suspected or primary diagnosis of NAIT were not tested or investigated, therefore most of these mothers are unaware of the risk the disorder poses and the potential to harm future pregnancies. Kamphuis, Paridaans, Porcelijn, Lopriore, and Oepkes (2014) states that mothers are often unaware of NAIT and the risk of foetal – maternal bleeding and ICH are high in a subsequent pregnancy. This is particularly important given the highly effective preventive measures that are currently available.

Of the seven suspected cases of NAIT, one had a moderate and the other two had mild thrombocytopenia. This finding is similar to Murat (2017) who report that NAIT can cause moderate and mild thrombocytopenia. Since it is anticipated that the majority of NAIT cases occurring in asymptomatic thrombocytopenic infants are overlooked, it is important to screen for suspected cases (Madani et al., 2012). However, the findings of the study show that all the suspected cases were never tested or investigated for NAIT. The possible reasons for not investigating the cases are mostly due to parents did not want to give blood or were ignored and never followed up.

However, in the majority of cases it is severe thrombocytopenia (< 50 x 109/L) caused by rare diseases such as NAIT that requires urgent investigation because neonates may present with, or develop, ICH which can result in long term neurological disability and even death (Carr et al., 2017; Roberts et al., 2008).

The reported incidence of NAIT is approximately 1 – 1.5 in 1000 live births. It is a relatively rare disease and identified as the most common cause of severe thrombocytopenia in neonates (Murat, 2017; Yougbare et al., 2018).

However, the incidence of NAIT maybe underestimated since there are not many studies conducted on the in utero foetal death, stillbirths and miscarriages. The incidence and mechanisms of how these deaths associated with NAIT are unknown (Yougbare et al., 2018).

Although several studies have reported that ICH is the most life – threatening outcome of severe NAIT (Meler et al., 2017; Refsum, Hakansson, et al., 2018), this study did not report any cases. These findings are not consistent with other retrospective studies with incidences such as those reported by Refsum, Hakansson, et al. (2018) at about six per 100 000 deliveries and Tiller et al. (2017) at 1 per 10 000 births.

A possible reason given by Refsum, Hakansson, et al. (2018) is that some cases of ICH due to NAIT were missed as the babies were initially asymptomatic. However, in this study it is likely that, unless the infants moved out of the area, they would have been re-admitted to Middlemore Hospital had they developed an ICH. These admissions would therefore have been detected by the study design since it spanned such a long period of time. Amongst babies with NAIT who also had affected siblings there is no record that any developed an ICH. It is reported that infants are more likely to be at risk of ICH if they had siblings who had ICH in a previous pregnancy (Strong & Eddleman, 2013).

With no cases of ICH found in this audit could be suggested that the incidence of ICH associated with NAIT is low in this study, on the contrary, many studies reported the serious consequences of ICH are neurological disorder and even death.

This could have been true if all suspected NAIT cases were investigated and screened for HPA.

Although HLA sensitisation is documented and reported to be associated with HPA alloimmunisation (Brouk, 2015), and can even cause severe thrombocytopenia, this study did not look for nor report any documented cases.

The possibility of HLA incompatibility again emphasises the value and importance of a screening program. Since NZBS Tissue Typing Laboratory performs the diagnostic tests to detect these antigens, it would be helpful to have HLA tested in women suspected of NAIT. In fact, Kjaer et al. (2017) and ADBH newborn clinic services (www.adhb.govt.nz, n.d) suggest that women who are HPA negative should be tested for HLA and for rarer maternal antibody types.

Moreover, it appears that the presence of HLA sensitisation in a woman increases the risk of HPA alloimmunisation. In addition, Peterson et al. (2014) and Dahl et al. (2017) document that multiparous women are more likely to be sensitised to HLA Class I antigens which can possibly cause NAIT in some neonates.

On the contrary, some studies fail to show an association between HLA antibodies and NAIT, and the role it plays in NAIT is controversial (Hutchinson et al., 2015; Meler et al., 2017).

However, the majority of studies document evidence for HLA – mediated NAIT. This is more obvious when the HPA antibodies are not detected. It is however reported that HLA antigens responds differently from HPA and do not have the same ability to induce thrombocytopenia (Gérald Bertrand & Kaplan, 2017; Kjaer et al., 2017; Meler et al., 2017).

Studies have demonstrated that maternal ABO group is associated with NAIT and could play a role in the risk of severity of NAIT due to anti – HPA – 1a (Ahlen et al., 2012).

Almost all mothers in this study who had infants with suspected or confirmed NAIT were ABO group O and Rhesus positive (O positive: O +). Although these results are

inconsistent with other studies, it is demonstrated that group O mothers can have infants who develop thrombocytopenia. Ahlen et al. (2012) reported that ABO group A mothers have a higher change of having a child with severe NAIT than mothers with group O. In addition, Peterson et al. (2013) and Crighton et al. (2017) agree that maternal ABO type plays a role in NAIT. Moreover, it is suggested that genetic properties of the maternal ABO genotype affect the immune response resulting in severe thrombocytopenia in the newborn (Ahlen et al., 2012).

The association of severe thrombocytopenia in NAIT and ABO antibodies needs confirmation and further studies need to be done.

All except one of the suspected or confirmed babies with NAIT were followed until the repeat platelet count resolved. The remaining infant was initially followed up but then discharged without the platelet count resolving.

NAIT is a transient and inert condition which remits spontaneously, and the rate at which the platelet count resolves depends on the rate at which the infant's circulation can remove maternal HPA antibodies (Bonstein & Haddad, 2017; Hoffbrand et al., 2005). The reason why some infants repeat platelet count resolved within 1 – 2 days is that most of the maternal HPA antibodies are cleared of the foetal circulation within 48 hours of age (Beachy, 2011).

This study found that the number of days the repeat platelet count resolved range from 1 day to 2 months. Similar results were obtained by Beachy (2011) and Peterson et al. (2013) that most courses of NAIT resolve by two weeks of age and the platelet count are normal by four weeks.

With rarity and the condition as self – limiting it usually remit spontaneously after repeat platelet count, but thrombocytopenia can persist for up to 6 weeks. In most infants with NAIT cases are not properly diagnosed or treated (Peterson et al., 2013).

In this study, most mothers with infants suspected or primarily diagnosed with NAIT were never tested therefore they may not have adequate records, yet it can be assumed that future pregnancies will be affected. Thus, it would be better to track and keep a record of mothers with infants suspected or diagnosed with NAIT so that they can be identified and be tested. This is to prevent any future siblings at risk of developing NAIT from suffering serious complications such as ICH (Brojer et al. (2016).

The guidelines and recommendations by Newborn Services Clinical Guidelines of Auckland District Health Board (ADHB) recommend that in any confirmed cases of NAIT, the future pregnancies should be referred to and managed by the Maternal Foetal Medicine Team (www.adhb.govt.nz, n.d).

Since the disease remits spontaneously, the advantages of investigating for NAIT and following up thrombocytopenic infants should be weighed against costs. In addition, considering the results of this study, the benefits of introducing routine screening for NAIT, with the increased costs involved, may not be justified.

In one of the suspected NAIT cases (number 209), the cord blood sample result showed mild thrombocytopenia (110 x 10^9/L) at birth. The infant was asymptomatic and discharged but readmitted after five days with a petechial rash. There was no bruising or bleeding noted. The repeat platelet count on day 6 was <10 x 10^9/L and the baby was transfused with platelets. Following transfusion, the repeat platelet count on day 7 was 32 x 10^9/L, still in the severe range, but after two weeks it had improved further, to a clinically safe level (62 x 10^9/L). The platelet count only fully resolved, however, after two months. There was no evidence of ICH on xray. This infant was first-born and there was no history of any problems in pregnancy; in particular no history of bleeding, and abnormal platelet count. The parents were not tested as part of the serological investigations for NAIT. The diagnosis was unclear from the records, but the working diagnosis was alloimmune thrombocytopaenia. This baby represents a case of delayed onset of presentation to the fifth postnatal day. Reiher et al. (2017) stated that evidence of HPA alloimmunisation might occur a few days after delivery, and therefore NAIT might be missed.

Usually infants present with symptoms such as petechiae and purpura at birth or few hours after birth, and blood results which show severe thrombocytopenia. Mothers typically have a good health history with no previous history of thrombocytopaenia, allo – and auto – immune disorders (Tiller et al., 2017).

Since there was no confirmation of the disorder and this baby was firstborn, if this was a case of NAIT then future pregnancies are likely to be affected. There should be a strategic plan for managing all suspected or confirmed cases so that they are followed up efficiently.

Several studies express concern that delayed or missed diagnosis of NAIT can result in devastating implications for subsequent pregnancies (Tiller et al., 2017).

The study findings show that most babies with suspected NAIT cases were not followed up. This can have disastrous medical consequences in the future pregnancies. Several studies have indicated that the advantage of having a screening program lies in having plans for managing the condition and an early antenatal management plan for mothers at risk (Abraham et al., 2018). In addition, the laboratory testing will be improved and able to identify the frequencies of HPA according to ethnicity. Improved technology assists identification of heterogenicity in the father whereby heterozygous means the chance of inheriting the antigen by the infant is 50%, whereas if homozygous it is 100%. Accordingly, clinicians usually recommend that mothers who are at risk deliver by caesarean section (Crighton et al., 2017; Peterson et al., 2014).

When the diagnosis is established, all known cases are recorded, and diagnosis of new cases allows for attempts at finding a compatible platelet donor. Studies have shown that affected infants should be monitored with planned management to avoid HPA alloimmunisation (Eyada et al., 2018).

However, not all screening programs are effective and there are limitations to the program. It is reported that cost – effective concerns are important in that only the most frequently occurring HPA implicated in NAIT for each local population are tested, while the other HPAs are not (Curtis, 2015).

Several studies have shown that establishing a screening program needs a proper setup such as methods of identifying foetuses at risk of bleeding, uncertainty of applying medical interventions and the cost of HPA typing reagents and equipment (Skogen, Husebekk, Killie, & Kjeldsen-Kragh, 2009). Many authors report that most women abstain or abscond from participating, while some move elsewhere, thus are lost to follow up (Kamphuis et al., 2014).

Since NZBS Tissue Typing laboratory is already conducting NAIT testing and there is a management protocol outlined by ADHB Newborn Clinical Services, there appears to be no need for a general screening program. However, it is wise to refer all suspected cases of NAIT for testing. On the other, it is significant to implement guidelines for investigating NAIT and setting guidelines by Middlemore Hospital.

Apart from screening or testing for HPA and NAIT, it is important to exclude platelet function defects, bleeding disorders, and conditions that affect the reticuloendothelial system (RES) among adults and neonates (Vadasz et al., 2015).

The findings of this study show that all ten cases of ICH occurred in preterm infants. ICH can occur in the presence of severe, moderate and mild thrombocytopenia, especially amongst preterm infants with low birth weight. The most common type of ICH was intraventricular haemorrhage (IVH), which occurred in more than 90% of cases. The remainder were either intraparenchymal, in 20%, or pituitary haemorrhage, in 10%. Amongst all types of ICH documented in the Middlemore study, just under one fifth of the infants died during or within a few hours of birth whereas the remaining four fifths had a normal outcome. Their thrombocytopenia resolved between or after few hours and one month after birth. The findings of the study are consistent with those reported by Ballabh (2010) and Tan, Svrckova, Cowan, Chong, and Mankad (2018) showing that IVH is a major dilemma for premature infants worldwide. Refsum, Meinke, et al. (2018) state that the incidence of intra- and periventricular haemorrhages are high among preterm infants. Factors most implicated as causing ICH in preterm infants are vascular fragility and haemodynamic instability as a result of injury after a hypoxic - ischaemic insult related to perinatal or postnatal illness (Carr et al., 2017).

Although there are no reported cases of ICH in term infants in this study, several studies report high incidences of intraparenchymal, subdural, and subarachnoidal haemorrhages (Kamphuis et al., 2014; Strong & Eddleman, 2013). The reason why these are not found or reported is beyond the scope of this study as it was limited to thrombocytopenia.

There are conflicting reports in regard to the association of thrombocytopenia with intraventricular haemorrhage (Gupta et al., 2011). However, this study found that IVH occurred in preterm infants with severe, moderate and mild thrombocytopenia. The results of the study do show that all the ICH cases were associated with thrombocytopenia. These findings are consistent with Brouwer et al. (2010) and Dahl et al. (2017) that thrombocytopenia was among the risk factors associated with ICH and essentially the most important predictor and causative mechanism of ICH.

With the incidence of NAIT in New Zealand still unknown, the incidence of ICH in affected babies cannot be predicted and requires further study. Furthermore, Refsum, Hakansson, et al. (2018) state that one of the key challenges in NAIT is that the exact

knowledge of its incidence and natural history is still unknown in populations without screening programs.

The estimated prevalence of ICH in thrombocytopenic infants is calculated to be 0.02% of the newborn population at Middlemore Hospital over a 11 - year study period from January 2005 to June 2016. With 11 cases of ICH reported in a total of 68910 births in the hospital, it is estimated that between 1 and 2 per 10,000 thrombocytopenic babies will be at risk of ICH. This prevalence is consistent with the published incidence of ICH which ranges between 0 – 10% (Becocci et al., 2018).

The incidence of ICH associated with NAIT cannot be discussed since there were no reported cases of ICH in suspected or confirmed NAIT in this study. Several studies have documented that ICH is found in 1 per 1000 term newborns (Kamphuis et al., 2014; Strong & Eddleman, 2013).

The reported incidence of NAIT maybe underestimated since not many studies are conducted on cases of in utero foetal death, stillbirths and miscarriages. The incidence and mechanisms of how these diseases are associated with NAIT are unknown (Yougbare et al., 2018).

It is possible that some cases of NAIT were missed in this study. Several possible reasons for this are discussed.

Over one third of term infants with severe thrombocytopenia do not have any cause documented. None of these cases was investigated for NAIT. Since there was no investigation of even the severe thrombocytopenia in these term infants there is a real chance that cases of NAIT were missed. It is recommended that NAIT should be excluded in infants who present with unexplained thrombocytopenia, especially severe thrombocytopenia, regardless of the presumed cause (Conti et al., 2014).

Of the 484 asymptomatic thrombocytopenic term infants, about 2.2% were severely thrombocytopenic and 10.0% moderate. Since there is no screening program in New Zealand such infants may not be further investigated. This could mean that there is a possibility that NAIT is missed which can affect the future pregnancies.

It would better to screen all term infants for severe thrombocytopenia in order to prevent any risk of ICH.

Furthermore, almost 90% of thrombocytopenic term infants were not followed up. A proper follow up protocol should be designed so that all severe, moderate and suspected cases of NAIT are followed up and investigated for a possible cause of the thrombocytopenia.

Apart from suspected cases of NAIT following an affected infant, most mothers are not investigated for possible NAIT even though they have a clinical history of stillbirths and miscarriages. Most causes of stillbirths are unknown since autopsies are declined and miscarriages are not investigated for possible NAIT.

Although stillbirths and miscarriages are recorded in the women who had asymptomatic thrombocytopenic infants, there were no recorded cases of NAIT amongst these women. It might be assumed that at least some of the stillbirths and miscarriages were due to NAIT. It would be helpful if all mothers' medical history is considered and screened for possible NAIT to prevent any future pregnancy complications.

Approximately 2% of the mothers of asymptomatic thrombocytopenic infants do not have any medical records. This includes five mothers with an unexplained thrombocytopenia. Some of the remaining mothers had a documented likely reason for their thrombocytopenia, and in some cases, this explained the thrombocytopenia in their baby (e.g. ITP). Thus, these were not possible cases of NAIT being missed.

Amongst the infants' siblings, the probable causes of their thrombocytopenia's were not documented in almost one third. Presumably this is because in most cases NAIT is only investigated in symptomatic infants and infants with family history of NAIT. Thus, most asymptomatic thrombocytopenic infants are not screened since the majority resolve spontaneously.

In general, an identifiable cause for the thrombocytopenia, including NAIT, was found in just over one fifth of all 794 study infants.

This study found that there is no correlation between NAIT and ICH in term infants. Although the most devastating effect of NAIT is ICH, none was documented in any of the confirmed or suspected cases. ICH was only detected in preterm infants, none of whom had NAIT. Although unlikely, due to the significant effect of ICH on a baby, it is possible that ICH may have been missed amongst early neonatal deaths or stillbirths.

Whilst the incidence of NAIT could not be determined by this study the estimated prevalence was determined and found to be very low. A prospective study of cases of NAIT would be valuable in New Zealand because it is a multicultural society, and HPA differ amongst ethnicities.

This study did not identify any association between NAIT and ICH. ICH was only detected in preterm infants. As has been discussed, the identification of cases of NAIT and appropriate management to prevent ICH is crucial.

Most neonatal deaths due to ICH were never investigated for NAIT. This may have been desirable. Moreover, women who had stillbirths were not investigated for NAIT and most causes of stillbirths are not recorded since the autopsies were rejected by the parents. It is, however, hospital policy for all recent stillborn infants to be examined by a paediatrician to detect any gross abnormality, and often there is a referral to the coroner (Meyer, n.d). It is advisable to investigate women who have a history of stillbirths and miscarriages and also to test for NAIT in stillbirth infants and their mothers.

The study findings show that most cases of thrombocytopenia, including NAIT, have a normal platelet between a few hours of birth and up to three months. This is in keeping with published data (Delbos et al., 2016). Ideally there should be follow up of thrombocytopenic infants and a repeat platelet count so that the true cause of thrombocytopenia is identified. However, this study shows that there appear to be very few problems in such babies, and most thrombocytopenia's resolve without incident.

The guidelines and protocol for managing NAIT are documented by ADHB for following best practice guidelines and are readily available on the internet. Apparently, Middlemore Hospital has its own management guidelines for cases of suspected NAIT (Meyer, n.d). These are less comprehensive than those of ADHB. It may be suggested that thrombocytopenia be routinely screened for and followed up since this study revealed its present in large numbers of well babies. However, it is hard to justify this in terms of cost and effort, since so few problems were detected in such babies.

The study findings show that most NAIT cases were not investigated and serological tests not performed. The possible reasons include parents being unwilling to undergo

blood tests and/or that an attempt to test for NAIT was not made or even that the healthcare worker was not aware of the availability or usefulness of HPA testing at Tissue Typing laboratory. There appeared to be an opinion amongst some neonatal staff that the test results for NAIT from NZBS were too slow in being received to be of value. A positive outcome of this study was the improved communication and updated awareness of neonatal staff regarding the timeliness and value of NAIT testing by NZBS. New forms were provided to the neonatal wards, and explanations given as to how to complete them.

It is considered best practice for every laboratory to establish its own reference ranges (Katsares et al., 2009). This study uses the Middlemore Hospital reference ranges for platelets (150 – 400 x 10^9/L); the site where all cord bloods FBC were performed. Since there are variations in reference ranges for platelet counts at different gestational ages, ideally Middlemore Hospital Haematology laboratory should produce its own age-appropriate reference ranges. Establishing a neonatal reference range for Middlemore Hospital Haematology laboratory would be very useful, since this region of New Zealand has a unique population, and such knowledge would be clinically useful, especially in defining the presence or absence of thrombocytopenia in preterm versus term babies.

Although this study audited a large number of cord blood FBC results, it did not show any significant findings. However, there was an attempt to determine the incidence but due to retrospective study incidence was not defined.

Since most thrombocytopenia cases resolve within short period time, it would be better to monitor the outcomes. Although cord bloods are used, peripheral should be collected to confirm the cause of thrombocytopenia.

Although many literatures have report that there are more preterm than term infants, this study found that there are more term infants than other gestation age group. On the other hand, there was no ICH detected in term infants with such a large data.

There are no specific reference ranges for preterm infants, however, the reference ranges used are same to that of the adult ranges.

Majority of studies have reported that NAIT occurs in 1 per 1000 live births however this study did not find any evidence of NAIT in almost over 50 000 asymptomatic babies. The number identified were small compared to other studies done.

The HPA antigens differ in different ethnicity populations therefore there is a need for ethnicity appropriate data on NAIT. There is a need for inclusion of broader specimen HPA antigen panels.

Limitations.

Since this study was conducted at Middlemore Hospital of Counties Manukau District Health Board (CMDHB) in Auckland City, New Zealand, with its unique demographic, the results may not be truly representative of the entire country. The overall prevalence cannot be over – emphasised. Not all cord blood samples were tested of the total birth registered therefore the true incidence of thrombocytopenia cannot be determined as this was a retrospective study. The prospective study to detect new cases of NAIT was a part of the study design, but unfortunately could not be conducted due to a lack of babies identified during the limited time frame. Being a retrospective study, information concerning possible aetiologies for the thrombocytopenia are incomplete.

The Concerto Database was used for gathering data and finding infants information, however, the audit identifies that only few cases of the information's were not recorded on the database. Permission for access to the database took longer than expected which shortened available time for collecting the data.

After the initial data collection, it was noted that the majority of the thrombocytopenic infants did not have a record of gestational age, birth weight, maternal history and siblings. Therefore, the Healthware database was used to collect the missing information. This incurred further delays.

Although two databases were used, not all information was recorded. This highlights a weakness in record-keeping, although in most cases the records were complete.

Most asymptomatic thrombocytopenic infants were discharged without any follow up platelet count done, however, few return to paediatrics ward after feeling ill due to other illness. The future study should follow up the cases from the thrombocytopenia through

to the time infant is discharge and readmitted after discharge for some reasons to determine the cause of the thrombocytopenia.

Whilst most asymptomatic infants did not have any information on the Concerto database except for the laboratory results, therefore they were categorised as asymptomatic. However, in a few cases they were found to be symptomatic after their NHI was obtained from the Healthware database. Thus, there were minor inconsistencies in records in both databases.

It would have been easier if all the required information was included in the Concerto database, but the long period retrospectively covered historical changes in databases as the hospital changed its record-keeping. In the future, hopefully researchers will find it easier to extract data from one source.

Most cases of asymptomatic thrombocytopenic infants were not followed up for examination or a repeat platelet count. There is no reason given for this, and it may be assumed in many cases that, because the cord blood FBC was a routine screening test, the health care worker wasn't even aware of the result when the infant was discharged after birth. Most of these cases were mildly thrombocytopenic although there were a few moderate cases.

In five cases of suspected NAIT cases (number 18, 26, 45, 209 and 242), the parents were not followed up, monitored or investigated for HPA antibodies. In these infants, the diagnosis was not well defined though the clinicians indicate that NAIT was the suspected diagnosis. Some of the terminologies used for the provisional diagnosis of NAIT include "working", "probable", "presumed" and "possible" NAIT. The reporting of the diagnosis should be well defined or standardised so that the records in the Concerto or Healthware databases are accurate should they be reviewed in the future. Whatever the definitions are used it would be better if all suspected cases of NAIT are tested for HPA antibodies so that the clinicians and parents are aware of the condition. If the cause is due to NAIT then the parents should be made aware of the medical implications in the future pregnancies.

Two cases of NAIT were given a primary diagnosis of NAIT, but they do not have any record on either the Healthware or Concerto databases. There is no indication whether their parents were tested or not. It would be better if NAIT is recorded as the primary diagnosis that there should be information about HPA presence and specificity,

particularly because of ethnic differences. With New Zealand being a multicultural country, it is helpful in finding compatible platelet donors and to prevent infants from HPA incompatibilities.

Without serological results, the true incidence of NAIT cannot be determined.

Since there is no screening program, most parents are not aware of the risk NAIT poses in future pregnancies where they have more than one child who also has severe thrombocytopenia. For example, in this audit, the same parents had twins who both had severe thrombocytopenia. In such cases, counselling should be provided to the parents regarding the risk of future pregnancies and the possibility of ICH or other serious complication in subsequent pregnancies. The parents should also be advised on the outcome of how the child will inherit the antigens from the father. For instance, in this case the father is homozygous for HPA – 1a, therefore almost all future pregnancies will be affected meaning there is 100% chance that the children will inherit HPA. There should be follow up on every confirmed NAIT cases to monitor and manage future pregnancies. The follow up policies and guidelines are already documented and published by the ADHB therefore I would only suggest that the guidelines should be implemented and followed in other district health boards. The recommendations and guidelines of managing future pregnancies are already available on www.adhb.govt.nz (n.d) for Newborn Services Clinical Guideline for NAIT and https://www.healthpoint.co.nz/ (n.d). In addition, I believe that Middlemore Hospital has guidelines already established, however, they are not published on the internet but the hospitals intranet.

In this study the suspected NAIT cases were neither monitored nor followed up even though subsequent pregnancies are at risk. However, since there are no ICH cases reported in the subsequent pregnancies, does it matter to monitor or follow up NAIT? Numbers of suspected and confirmed NAIT cases are small, however, therefore no conclusion should be drawn too quickly by stating that ICH is not associated with NAIT based on the study data. More data and research is required for future studies. The further study should be designed to determine the associations of ICH and NAIT.

The other problem identified is that sometimes parents refused to give a blood sample and so suspected cases were never tested. Kamphuis et al. (2014) state that some of the main reasons for no follow up tests are the refusal of the parents to give blood samples,

work overload by the nurses, and technical problems such as a clotted blood sample. Due to the rarity of the disease, many clinicians disregard the disease and symptoms such as bruising could be regarded as due to birth trauma.

Testing at NZBS may miss antibodies to HPA with high frequency in other ethnicities.

The NAIT testing is performed at the Tissue typing laboratory at NZBS and if these HPA were not tested then there is possibility that the antibodies could be missed. Therefore, I would suggest a broader ethnic HPA panel to be included in the panel.

Of the total sample processed (n = 66109), about 6.1% of the infant's test results were excluded from this study since they were clotted or clumped sample which the results are not conclusive.

Approximately, 4.1% were not processed although they were registered as total number of births at Middlemore Hospital.

Therefore, the percentage of infants that provide conclusive results were about 90% of the total sample process that include normal results, thrombocytopenia and thrombocytosis.

As part of the quality control measures all clumped or clotted samples should be followed up so that the results are conclusive. A protocol should be designed so that the neonatal intensive care units or birthing units follow up infants with clotted or clumped samples since any abnormal results can be overlooked and not managed appropriately.

Recommendations

The databases should include all patient's information so that the data searches are done without any constraints.

The study found that most asymptomatic infants were not followed up. This could be due to the recommendations and guidelines already set by Newborn Services Clinical Guideline for NAIT by ADHB and New Zealand Maternal Foetal Medicine Network (NZMFMN) – Auckland. Since all guidelines and policies are in existence, I would suggest that all district health boards to incorporate plans to monitor all NAIT cases and be tested. This will assist in identifying the incidences of HPA in different ethnicities.

Although most studies have indicated that cost of establishing a screening program is cost effective, it can be achieved in New Zealand since all the policies and guidelines are already in existence and NAIT testing is conducted at the Tissue Typing Laboratory at NZBS. However, not all screening programs are effective and there are limitations to the program. It is reported that cost – effective is a major concern, meaning only the most frequently occurring HPA that implicated NAIT in each ethnicity population are tested while the other HPAs are not tested (Curtis, 2015).

This study found that most suspected NAIT cases were not tested and considering the life – threatening consequences of the conditions, I would suggest that all suspected cases should be investigated for HPA.

Establishing a general screening program would not be an option, but given the policies and guidelines already published and well – established laboratory testing HPA, I would recommend that all suspected cases of NAIT or those with family history of NAIT should be screened and investigated.

Since New Zealand is a multicultural country it would be better to screen every woman whose infant develop severe thrombocytopenia and are at risk of NAIT. The possibility of a screening program is eminent given the fact that NAIT is being tested at the Tissue Typing laboratory, and the polices and guidelines are documented and updated by the ADHB newborn service clinic and Middlemore Hospital intranet.

Firstly, the testing for NAIT is already established at the Tissue typing laboratory at NZBS, therefore using diagnostic criteria for NAIT all thrombocytopenic (asymptomatic and symptomatic) infants suspected of NAIT and family history of NAIT should be tested to exclude NAIT from other causes of thrombocytopenia.

Secondly, the clinical guidelines for neonatal alloimmune thrombocytopenia are already created by the ADHB Newborn Service Clinical and CMDHB intranet with a given policies and guidelines it would be better if district adopted the policies and implement it.

With policies and guidelines already created, all district health board should gazette the hospital neonatal intensive care units and birthing units to refer all suspected cases for investigation at the Tissue Typing laboratory at NZBS.

Thirdly, according to the Ministry of Health New Zealand, most maternity – related services are free and subsidised. The pregnant woman can have free access to healthcare facilities with the evidence they are permanent New Zealand residence. The funding is to provide the parent with better health care so that the baby will be as healthy as possible. The information can be accessed at www.health.govt.nz (n.d.). Given the fact that health care for the babies are funded, I would suggest that screening suspected NAIT is an alternative option.

The next task is to screen all suspected NAIT to establish the frequencies of HPA in different ethnicity population. The importance of this to detect HPAs that rare and low frequency so that platelet alloimmunisation is avoided.

With already published frequencies of HPA in different ethnicity population by Newborn Services Clinical Guideline for NAIT by ADHB it would be helpful if all these HPAs are tested at the laboratory. Not all women will be positive HPA therefore with screening and investigation will exclude the NAIT from other cases. However, consideration should be taken in account that rare or low frequency HPA do exist.

Identifying and investigating suspected NAIT is to prevent any risk the disease pose. Most devastation cause in NAIT is ICH, and screening will reduce the risk thus preventing the risk of neurological sequelae and even death in the future pregnancies.

Although ADHB updated a list of possible HPA alloimmunisation in different ethnicity population, and to the best of my knowledge, there has never being a study done on the prevalence and incidence of NAIT in New Zealand. Give the reason that no study is done, it would be helpful if all the suspected cases are tested and a study conducted to determine the real incidence and prevalence of NAIT in New Zealand. As my study is centred in Middlemore Hospital, Counties Manukau District Health Board, therefore I cannot generalise and compare my findings to the general population of New Zealand.

This study found that more than half (about 66%) of the thrombocytopenia infants were not followed up. The neonatal wards may have their own follow up and management of thrombocytopenia infants, I would suggest that a ward adopt and draft a treatment and management guideline for all thrombocytopenia infants.

In comparison to other centres Counties Manukau has a high birth rate, and they vary in different ethnicity populations. In the 2016 and 2017 report of Women's Health and

Newborn Annual Report by Counties Manukau Health reported the percentage of women who live at the district with 27.9% were Pacific Islanders, 26.3% were New Zealand Europeans, 19.8% were Maori, 11.0 were Indians and 8.6% were Chinese (www.countiesmanukau.health.nz, n.d). With such a high demand on the maternity, child and youth health services it can results in staff over worked and stress. This could mean work efficient decrease in thereby lack of better service provided by the staff which can result in ignoring some diseases that are rare.

Although these results show significant findings, I would suggest that general screening is not an option but screening all suspected cases of NAIT would be a better option. There is a need for more study and awareness before it can be established.

Future research

In many countries, establishing a screening program for NAIT is difficult given the reasons as lack of a suitable population statistics on prevalence of severe thrombocytopenia and episodes of bleeding problems; and a consistent approximate estimate of the prevalence and severity of NAIT of the overall population is needed.

In most cases of NAIT, the screening is performed only on the symptomatic infants or after the infants develops the symptoms after or during birth. The cases where the infants are asymptomatic are not screened or are referred for NAIT testing. However, due to the rarity of the disease, in many cases are ignored and assumed that the symptoms are due to physical trauma caused during the birth.

The future studies should review all severe thrombocytopenia infants and screened for HPA including their parents. The aim of the prospective study was designed to identify NAIT cases but due to the time frame of the study, it was never conducted.

The outcome of this study can help determine good record keeping, managed thrombocytopenia infants appropriately, and identify foetuses or neonates at risk of NAIT. The most essential elements in identifying foetus or neonates at risk of NAIT is to prevent the risk the disease pose and the burden of the disease in subsequent pregnancies (Kamphuis et al., 2014).

Establishing the true prevalence in a population is identifying the potential infants and the mothers at risk of NAIT. The mothers with known NAIT cannot justify the true incidence of NAIT (Kamphuis et al., 2014), therefore it is appropriate to refer suspected

NAIT cases for serological studies to confirm the diagnosis or exclude other causes of the thrombocytopenia.

Conclusion

The aim of the study was to determine NAIT cases in asymptomatic term infants. However, there was no evidence of NAIT in asymptomatic term babies. The study found almost none in over 50 000 babies.

The findings suggest and assume that for every 10000 births, one newborn will develop NAIT, and of the conclusive test results, we estimate a prevalence of 128 infant will develop thrombocytopenia for every 10 000 births.

To the best of my knowledge this is the first study conducted on NAIT in New Zealand, however, there are more limitations of the study therefore I recommend that proper study be designed to determine the incidence of NAIT in New Zealand.

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