FACTORS INFLUENCING PLATELET COUNT INCREMENT AFTER PLATELET TRANSFUSION AMONG THROMBOCYTOPENIC PATIENTS IN HOSPITAL USM

BY

DR MOHD ZAMRI BIN MOHD HAYAT

Dissertation Submitted In Partial Fulfillment Of The Requirements

For The Degree Of Masters Of Pathology (Hematology)

UNIVERSITI SAINS MALAYSIA

NOVEMBER 2016

ACKNOWLEDGEMENT

All praises to Allah S.W.T the Most Merciful and the Most Beneficent.

First of all, I would like to express my thankfulness to Allah S.W.T for His blessing and for giving me strength and ability that finally I have managed to complete my research successfully within the given time.

Here, I wish to take this opportunity to express my gratitude and appreciation to my helpful and knowledgeable supervisor, Dr Mohd Nazri Hassan, whom I deeply indebted for his continuous supervision and care in the process of finishing this study. Without his good suggestion and well given encouragement, I would never be able to write this dissertation completely. Thank you so much for your commitments and your active participation in helping me throughout this study.

With great pleasure, I would like to acknowledge Assoc. Prof Suhiar Abbas, my co-supervisor for their helps, ideas and continuous support. Also not to forget, a million thanks to all staff in transfusion medicine unit, hematology department staff and record unit who involves direct or indirectly in this study process.

Most importantly, my special appreciation goes to my beloved wife, Nur Azimah Yusof, my daughters Nur Insyirah Liyana and Nur Inas afina for their sacrifice, countless loves, strong faith, understanding and patience throughout my study. Without their support, I will not be able to perform as I am right now. Not to be forgotten, also to my parents who raised me with

a love of knowledge, my parent in law and the rest of my family members for their prayers, encouragements and endless supports.

TABLE OF CONTENTS

Contents		*Page*
1.	**Acknowledgement**	**ii**
2.	**Table of contents**	**iv**
3.	**List of tables**	**vii**
4.	**List of figures**	**viii**
5.	**List of abbreviations**	**ix**
6.	**Abstract (in Malay Language)**	**xi**
7.	**Abstract (in English)**	**xiii**
8.	**Chapter1: Introduction**	**1**
9.	**Chapter 2: Literature Review**	**4**
	2.1 Platelet concentrates	**5**
	2.2 Indication of platelet transfusion	**7**
	2.3 Platelet selection for transfusion	**14**
	2.3.1 ABO compatibility	**14**
	2.3.2 Rhesus-D (RhD) compatibility	**17**
	2.3.3 Irradiated platelet concentrates	**18**
	2.3.4 Cytomegalovirus-seronegative platelet concentrates	**19**
	2.3.5 Leucodepleted/ leucoreduced platelet concentrates	**20**
	2.3.6 Platelet transfusion for neonates	**20**
	2.4 Monitoring response to platelet transfusion: Calculation of platelet recovery and corrected count increment	**21**
	2.5 Factors influencing post-transfusion platelet increment	**23**
	2.5.1 Platelet factors	**23**

2.5.1(a) ABO compatibility platelet transfusion **23**

2.5.1(b) Rhesus-D status with post-transfusion platelet increment **24**

2.5.1(c) Type of platelet concentrates (Apheresis platelet versus random platelet) **25**

2.5.1(d) Age of platelet concentrates **25**

2.5.1(e) Leucoreduced platelet concentrates **27**

2.5.1(f) Pathogen reduction platelet concentrates **28**

2.5.1(g) Irradiated platelet concentrates **29**

2.5.2 Patient and disease factors **30**

2.5.2(a) Age, gender, height and weight of patient **30**

2.5.2(b) Splenomegaly **31**

2.5.2(c) Disseminated Intravascular coagulopathy **31**

2.5.2(d) Infection **32**

2.5.2(e) Malignancy **33**

2.5.2(f) Concomitant disease **34**

2.6 Platelet refractoriness **35**

. 2.6.1 Definition **35**

2.6.2. Factors associated with platelet transfusion refractoriness **36**

10. Chapter 3: Research Objectives **38**

3.1 General objective **39**

3.2 Specific objective **39**

3.3 Benefit of the study **39**

3.4 Rational/Justification of the study **40**

. 3.5 Research question/problem **40**

11.	**Chapter 4: Research Methodology**	**41**
	4.1 Study design	**42**
	4.2 Sampling for cases	**42**
	4.3 Sample size calculation	**43**
	4.4 Sampling method	**46**
	4.5 Data collection	**46**
	4.6 Post-transfusion platelet increment calculation	**47**
	4.7 Data entry and statistical analysis	**51**
12.	**Chapter 5: Results**	**52**
13.	**Chapter 6: Discussion**	**61**
14.	**Chapter 7: Limitations**	**77**
15.	**Chapter 8: Conclusions**	**79**
16.	**Chapter 9: References**	**81**
17.	**Appendices**	

LIST OF TABLES

		Page
Table 2.1	Comparison of platelet components produced from whole blood or by apheresis	**7**
Table 2.2	Some causes of thrombocytopenia (excluding condition that usually causes pancytopenia)	**8**
Table 2.3	Indication for platelet transfusion	**11**
Table 2.4	Suggested platelet transfusion thresholds for neonates	**12**
Table 2.5	Definition of ABO-matched transfusion	**16**
Table 2.6	ABO mismatches classification	**16**
Table 2.7	Indication for irradiated platelets	**18**
Table 2.8	Factors associated with platelet transfusion refractoriness	**37**
Table 4.1	Definition of ABO compatibility	**47**
Table 5.1	Patients demographic and descriptive analysis	**52**
Table 5.2	Descriptive analysis of platelet concentrates usage parameters	**55**
Table 5.3	The association of patient and disease factors with poor post-transfusion platelet increment by Simple Logistic Regression	**56**
Table 5.4	The association of laboratory factors with poor post-transfusion platelet increment by Simple Logistic Regression	**57**
Table 5.5	Factors influencing of post-transfusion increment	**58**
Table 5.6	Incidence of platelet refractoriness	**59**
Table 5.7	Risk factors of platelet transfusion refractoriness	**60**

LIST OF FIGURES

		Page
Figure 2.1	Approach for platelet transfusion refractoriness	**13**
Figure 4.1	Flowchart of the research methodology	**50**
Figure 5.1	Descriptive statistic of clinical factors that were analyzed in this study for CCI	**54**

LIST OF ABBREVIATIONS

AML	Acute myeloid leukaemia
BSA	Body surface area
BV	Blood volume
CCI	Corrected count increment
CI	Count increment
CMV	Cytomegalovirus
CNS	Central nervous system
CPD	Citrate phosphate dextrose
DIC	Disseminated intravascular coagulation
GP	Glycoprotein
GVHD	Graft-versus-host disease
Gy	Gray
hr	Hour/ hours
HLA	Human leucocytes antigen
HPA	Human platelet antigen
USM	Universiti Sains Malaysia
KIV	Keep in view
LR	Likelihood ratio
mL	Millilitres
MLR	Multiple logistic regression
OGDS	Oesophagogastroduodenoscopy
PD	Platelet dose
PDN	Pusat Darah Negara
PI	Platelet increment

PPR	Percentage/ post-tranfusion platelet recovery
PR	Platelet recovery
PRA	Panel Reactive Antibody
PR-PLT	Pathogen reduced platelet
Rh	Rhesus
RhD	Rhesus D
ROC	Receiver operating characteristic
SLR	Simple logistic regression
UK	United Kingdom
WBC	White blood cell

ABSTRAK (MALAY LANGUAGE)

FAKTOR-FAKTOR YANG MEMPENGARUHI KENAIKAN KIRAAN PLATELET SELEPAS TRANSFUSI PLATELET PADA PESAKIT TROMBOSITOPENIK DI HOSPITAL USM

Banyak kajian telah dijalankan untuk menentukan faktor-faktor yang mungkin menyumbang kepada kenaikan platelet pasca transfusi. Bagaimanapun, tiada lagi data yang didokumenkan boleh didapati dalam institusi kami dalam bidang pengetahuan ini. Oleh itu, kajian ini telah dijalankan untuk menentukan faktor-faktor berkaitan transfusi yang boleh mempengaruhi kenaikan platelet selepas transfuse pada pesakit trombositopenik.

Kajian kohort kebelakang ini melibatkan 97 pesakit yang menerima 351 transfusi platelet di Hospital Universiti Sains Malaysia dalam tempoh Januari 2013 hingga Disember 2014. Kiraan platelet pada pra dan pasca telah diukur dengan menggunakan penganalisa hematologi Sysmex XE5000 dan kenaikan kiraan yang diperbetulkan ("CCI") telah dikira pada 1 jam dan 24 jam selepas transfusi. Kenaikan lemah ditakrifkan sebagai "CCI" < 7500 $m^2/\mu L$ pada 1 jam dan "CCI" < 4500 $m^2/\mu L$ pada 24 jam. Faktor-faktor yang dianalisis adalah kanser, demam, DIC, pendarahan, splenomegali, jenis platelet pekat (rawak berbanding aferesis), keserasian ABO dan usia platelet pekat. Regresi logistik mudah ("SLR") dan berganda ("MLR") telah digunakan untuk analisis statistik dan nilai $p < 0.05$ dianggap sebagai bernilai.

Kebanyakan pesakit adalah Melayu (92.8%) dengan umur median 47 tahun. Bilangan pesakit lelaki dan perempuan adalah hampir sama. Kebanyakan pesakit adalah O positif (40.2%), diikuti oleh B positif (27.8%), A positif (26.8%) dan AB positif (5.2%). Min dan median CCI

adalah masing-masing 15,834.54 $m^2/\mu L$ dan 10, 350 $m^2/\mu L$. Peratusan pesakit mendapat CCI bagus adalah 66.1%.

"SLR" dan "MLR" menunjukkan hanya "DIC", umur platelet dan keserasian ABO adalah faktor-faktor bernilai yang mempengaruhi kenaikan platelet pasca transfusi. DIC mendapat masing-masing kemungkinan terlaras 5.31 kali dan 4.41kali bagi "CCI" platelet pasca transfusi lemah (nilai-$p < 0.001$, 95%CI=3.06,9.24), dan kerefraktoran platelet transfusi (nilai-$p < 0.001$, 95%CI=2.0,9.7). Selain itu, umur platelet > 3 hari mendapat kemungkinan terlaras 2.2 kali "CCI" lemah berbanding dengan segar platelet (nilai-$p = 0.004$, 95% CI = 1.29, 3.75). Platelet dengan keserasian ABO mendapat 80% ketidakmungkinan terlaras untuk mendapatkan "CCI" lemah pasca transfusi berbanding dengan platelet serupa ABO (nilai-$p = 0.011$, 95%CI=0.06,0.65). Faktor-faktor klinikal dan makmal lain tidak bernilai secara statistik.

Kesimpulannya, pesakit dengan "DIC" dan menerima platelet pekat berusia ≥4 hari akan memerlukan lebih banyak platelet transfusi dalam mencapai kenaikan platelet yang dikehendaki berbanding dengan pesakit tanpa "DIC" dan yang menerima transfusi ≤3 hari platelet pekat. Di samping itu, pesakit yang menerima platelet pekat serasi ABO mungkin memerlukan jumlah yang lebih sedikit transfusi platelet berbanding ABO serupa dalam mencapai tahap platelet sasaran.

ABSTRACT (ENGLISH LANGUAGE)

FACTORS INFLUENCING PLATELET COUNT INCREMENT AFTER PLATELET TRANSFUSION AMONG THROMBOCYTOPENIC PATIENTS IN HOSPITAL USM

Many studies had been performed to determine the factors that may contribute to post-transfusion platelet increment. However, no documented data is available in our institution in this area of knowledge yet. Therefore, this study was conducted to determine the transfusion-related factors that may influence post-transfusion platelet increment in thrombocytopenic patients.

This retrospective cohort study involved 97 patients who received 351 of platelet transfusions in Hospital Universiti Sains Malaysia within the period of January 2013 till December 2014. The pre and post transfusion platelet counts were measured by using Sysmex haematology analyzer XE5000 and the corrected count increment (CCI) was calculated at 1 hour and 24 hours post transfusion. Poor increment was defined as CCI<7500 $m^2/\mu L$ at 1 hour and CCI<4500 $m^2/\mu L$ at 24 hours. The analyzed factors included, underlying malignancy, fever, DIC, bleeding, splenomegaly, type of platelet concentrates (random versus apheresis), ABO compatibility and age of platelet concentrates. Simple (SLR) and multiple logistic regression (MLR) were used for statistical analysis and *p*-value of < 0.05 considered as significant.

Most patients were Malays (92.8%) with a median age of 47 years old. Male and female patients were nearly equal in number. Majority of patients were O positive (40.2%) followed by B positive (27.8%), A positive (26.8%) and AB positive (5.2%). The mean and median of

CCI was 15,834.54 $m^2/\mu L$ and 10,350$m^2/\mu L$, respectively. The proportion patients with good CCI was 66.1%.

SLR and MLR showed that only DIC, platelet age and ABO compatibility were significant factors influencing post-transfusion platelet increment. DIC had 5.31 and 4.41 time adjusted odds for poor post-transfusion platelet CCI (*p*-value<0.001, 95%CI=3.06,9.24) and platelet refractoriness (*p*- value <0.001,95%CI=2.0,9.7), respectively. Meanwhile, platelet age of >3 days had 2.2 time adjusted odds of poor CCI compared to fresh platelet (*p*-value = 0.004, 95% CI=1.29,3.75). ABO compatible platelet transfusion had 80% adjusted odds of unlikelihood to get poor post-transfusion CCI compared with ABO identical platelet (*p*-value=0.011, 95%CI=0.06,0.65). Other clinical and laboratory factors were not statistically significant.

In conclusion, patients with DIC and who receive day ≥4 platelet concentrates will need more platelet transfusions to achieve the desirable platelet increment compared to non DIC patients and who receiving day ≤3 platelet concentrates patients, respectively. In addition, patients who receive ABO compatible platelet concentrates may need fewer platelet transfusion than ABO identical in achieving the similar target platelet level.

Chapter 1

Introduction

1.0 INTRODUCTION

Background of the study

Platelets are one type of blood cells which are mainly involved in circulation hemostasis. Platelets are anuclear and there are also known as thrombocytes. They are derived from megakaryocytes cytoplasm fragments which are situated in the bone marrow and then these end products are released to peripheral circulation (Machlus *et al.*, 2014) . A megakaryocyte can give rise to 1000-5000 platelets and normally these newly produced platelets are able to survive in circulation up to 10 days (Kaushansky K, 2004). It had been estimated that by physiological process, a normal adult will have daily platelet loss of about 7 x 10^9/L (Hanson and Slichter, 1985) but in malignancy and severe infection the losses will increase due to higher platelet demand (Schiffer *et al.*, 2001).

Platelet function has been well established for its involvement in stopping any bleeding at the site of interrupted endothelium. They clump together at the site of injury and platelet plug is then developed at the injured site. Initially, platelets will attach to substances outside the interrupted endothelium then adhesion will take place. Then, they change shape, turn on receptors and secrete chemical messengers and lastly, they connect to each other through receptor bridges in a process which is called as aggregation (Yip *et al.*, 2005).

Platelet concentrates as important tools in treating most cases of thrombocytopenia cannot be denied. Most clinicians are very keen to transfuse this type of blood component for most thrombocytopenic patient with bleeding condition or high risk patient for bleed. However, the dilemma for most clinicians and haematologists is their inability to reach desirable post

transfusion platelet count increment even after standard calculations for such transfusions have been applied.

Many factors have been suggested to influence the corrected count increment of platelet transfusion. A study done by Shamee Shatry et al showed that presence of splenomegaly and use of antiplatelets gave significant refractoriness in platelet transfusion (Shastry and Chaudhary, 2012). Meanwhile, another study found that patients who received ABO-identical platelet transfusions had a better increment compared to those who received ABO-compatible platelets but incompatible plasma (Heal *et al.*, 1993).

Even though many factors had been proposed by many studies that may influence those increments, specific prediction of increment for each patient for platelet transfusion is quite troublesome. Most patients may have multiple factors and each factor has its own weightage in post-transfusion platelet increment. A few factors that also need to be considered are fever, bleeding, disseminated intravascular coagulation (DIC), age, gender, type of platelet concentrates and ABO compatibility.

Since there is no local data available pertaining to this issue, it is hoped that the results of this study can be used to evaluate the factors which may contribute to post platelet transfusion increment in Hospital Universiti Sains Malaysia (USM) thrombocytopenic patients. The result of this study can also be used as a reference data for future use by haematologists and clinicians among the criteria to be considered prior to transfusion.

Chapter 2

Literature Review

2.0 LITERATURE REVIEW

2.1 Platelet concentrates

Platelet concentrates can be prepared from whole blood donation (random platelet) or single donor apheresis (apheresis platelet). Platelet concentrates derived from whole blood donation are produced by using different sequential centrifugation steps. Selected whole blood donations are centrifuged (hard spin) to separate plasma and red blood cells from the buffy coat layer containing leucocytes and platelet. Then, the buffy coats will undergo second centrifugation (soft spin) to separate leucocytes and residual red blood cells from the platelets. The remaining platelet are suspended in a mixture of additive solution and plasma. Blood separating device are needed for production of single donor platelet apheresis (Holbroa *et al.*, 2013).

By comparison, random platelets show less yield of platelet count per mL compared to apheresis platelet concentrates. Pusat Darah Negara (PDN) has stated that 60-70mL of random platelets contain $>5.5 \times 10^{10}$ platelets and a 250-300 mL of apheresis platelet concentrate contains more than 2×10^{11} platelets. Typically, 5-10 units of random platelets are used for 1 adult dose of platelet transfusion, meanwhile one-unit apheresis is equal to 5-6 unit of platelet random in term of platelet concentration (PDN, 2015).

Each unit of random platelets concentrate should increase the platelet count 5000 to 10,000/μL in the typical 70-kg human. Pools of 4 to 6 units, will contain roughly 3×10^{11} platelets and should give a platelet increment of 20,000 to 60,000/μL (Denise M. Harmening, 2005). Nevertheless, in this modern preparation process, apheresis platelets, and platelet rich plasma

or buffy coat platelet concentrates have similar concentration ≈ 1.5 x 10^9/mL (Heal and Blumberg, 2004).

Most of worldwide blood bank centers have used Standard for Blood Banks and Transfusion Services, 26th edition or American Association of Blood Banks 2009 guideline or Guide to the preparation, Use and Quality Assurance of Blood Component 16th edition as their reference bodies to regulate the quality of platelet component products. Thus, either random or apheresis platelet concentrates preparation should follow and maintain its quality standard according to the above regulations with minimal platelet content of >2.4 x 10^{11}/ unit and the number of residual leucocytes and red blood cells of <1 x 10^6/unit and 5 x 10^9/unit, respectively (Holbroa *et al.*, 2013).

The prepared platelets can be stored up to 7 days with good viability if good standard operating procedures have been applied, such as storage at 20^0C to 24^0C in citrate phosphate dextrose (CPD) or CDP-adenine solution with agitation motion (Cardigan and Williamson, 2003). However, in Malaysia, it has been practiced that platelet storage is up to 5 days as following the PDN guidelines which state that the shelf life for any platelet concentrate is 5 days from collection date (PDN, 2015).

Since earlier days, it has been accepted that platelet concentrates are grouped according to their ABH antigens as A and B blood group antigens have been shown to exist on the platelet membrane (Aster, 1965). Furthermore, ABO mismatches were recently found to have some adverse effects on platelet transfusion efficacy and even toward their recipients themselves (Cid *et al.*, 2013).

In our centre, we use Trima Accel and Haemonetic MCS+ 9000 as apheresis devices and both are licensed for use. Both types of platelet concentrates have their own advantages and disadvantage as illustrated below based on Holbroa et al (Holbroa *et al.*, 2013) (Table 2.1).

Table 2.1: Comparison of platelet components produced from whole blood or by apheresis.

Platelet product	Advantages	Disadvantages
Random platelet	1. Avaibility 2. Platelet dose modification 3. Avoids product waste 4. No additional donor risk	1. Multiple donor exposure 2. Difficult human leucocyte antigens (HLA)/ human platelet antigens (HPA) matching.
Apheresis platelet	1. Less donor exposure 2. Automation and standardization 3. HLA/HPA matching	1. Donor availability 2. Higher production cost 3. Limited platelet dose 4. Donor risk from apheresis procedure.

(Adapted from (Holbroa *et al.*, 2013))

2.2 Indications of platelet transfusion

In general, platelet transfusion is used for the purpose of preventing or treating bleeding condition due to thrombocytopenia caused by non-immunologic causes, immunological causes or ablative treatment. Table 2.2 shows some examples of causes of thrombocytopenia (Bain, 2015). Most institution set a standard of platelet transfusion as prophylactic transfusion at threshold level of $\geq 20 \times 10^9$ /L platelets. In addition, platelet transfusions continue to be used to treat active bleeding as required, independent of platelet levels based on specific patient needs and this action is called therapeutic platelet transfusion. A previous study reported that eight-fold increased risk of bleeding with platelet level below 5×10^9/L and a two-fold increased risk with platelet count between 5 to 15×10^9/L compared to previously reported figure of 20 to 29×10^9/L (Webert *et al.*, 2006).

Table 2.2: Some causes of thrombocytopenia (excluding conditions that usually causes pancytopenia).

Failure of platelet production	
Congenital (inherited or resulting from intra-uterine events)	May-Hegglin anomaly, Sebastian syndrome, Bernard-Soulier syndrome, megakaryocytic hypoplasia, reticular agenesis, placental insufficiency, hemolytic disease of newborn
Inherited but not present at birth	Fanconi anaemia
Acquired	Anticancer drugs, thiazide, myelodysplastic syndrome, HHV6 infection, interferon therapy, paroxysmal nocturnal hemoglobinuria, alcohol abuse, anorexia nervosa, autoimmune acquired amegakaryocytic thrombocytopenia, graft-versus-host disease, hypervitaminosis A, copper deficiency, arsenic poisoning.
Increased platelet consumption, destruction or removal-immune mechanism	
Congenital	Alloimmune thrombocytopenia (transplacental transfer of maternal auto/alloantibody), maternal drug hypersensitivity
Acquired	Autoimmune thrombocytopenic purpura (e.g. systemic lupus erythematosus, Evans syndrome), alloimmune (e.g. transfer of donor lymphocytes during stem cell transplantation, infusion of plasma containing platelet alloantibody), drug-induced immune thrombocytopenia (e.g. protamine-heparin thrombocytopenia), drug-induced autoimmune thrombocytopenia (e.g. gold salt), food-associated immune thrombocytopenia (e.g. tahini, *lupines termis* beans), immune thrombocytopenia associated with infection (e.g. HIV, cytomegalovirus, mycoplasma pneumoniae), post-transfusion purpura, cocaine abuse
Non-immune mechanisms	
Congenital	The Schulman-Upshaw syndrome, hereditary phytosterolaemia, Kaposiform haemangioendotheliaoma, intrahepatic infantile haemangioma, Type IIB vWF disease.
Acquired	DIC, thrombotic microangiopathy, post-transplant hepatic veno-occlusion disease, viral hemorrhagic fevers, Rickettsial infection, certain bacterial and protozoal infection, extracorporeal circulation, peripheral blood stem cell apheresis, massive transfusion, Kaposi sarcoma, M-CSF, snake bite.

"Table 2.2. Continued"

Redistribution of platelets	
Congenital	Hypersplenism
Acquired	Hypersplenism (including acute sequestration in sickle cell disease), hypothermia.
Uncertain or complex mechanisms	
congenital	Extreme prematurity, Wiskott-Aldrich syndrome, Grey platelet syndrome, Chediak-Higashi anomaly, Jacobsen syndrome, certain inborn errors of metabolism, Pearson syndrome
Acquired	Phototherapy in the neonate, respiratory distress syndrome, neonatal herpes simplex infection, neonatal hyperthyroidism, miliary tuberculosis, Grave disease, hypothyroidism, pregnancy-associated thrombocytopenia, Wilson disease.

(Adapted from(Bain, 2015))

European and American guidelines, are recommending the use of a threshold of 10 x 10^9/L as recommendation level for transfusion in patients with acute leukemia and chemotherapy-induced cytopenia. In addition, in patients with no fever, the acceptable threshold is even lower, up until 5 x 10^9/L but in presence of fever the trigger level for transfusion is usually recommended at 20 x 10^9/L ,whereas platelet counts > 50 x 10^9/L is recommended before surgery or invasive procedures (lumbar puncture, epidural anaesthesia, gastroscopy, and biopsy, insertion of indwelling lines, transbronchial biopsy, liver biopsy, laparotomy or similar procedures), massive transfusion, disseminated intravascular coagulation and cardiopulmonary bypass (British Committee for Standards in Haematology, 2003).

Meanwhile, platelets should be maintained at 100 x 10^9/L in surgical procedure at critical site (brain or eyes), multiple trauma or central nervous system injury. Lower threshold of 10 x10^9/L for prophylactic transfusion is also recommended for certain conditions such as in hematopoietic stem cell transplantation and chronic stable thrombocytopenia (Haematology and Force, 2003; Centre, 2007).

In our local guidelines for platelet transfusion, we use Pusat Darah Negara guidelines as our local reference whereby the transfusion threshold level is based on clinical conditions, as summarized in Table 2.3 (PDN, 2007). However, PDN guidelines have not included peadiatric patients specifically. Thus, Guidelines for platelet transfusion in pediatric patients created by Department of Paediatrics, St Mary's Hospital, London, United Kingdom (UK) can be used in other centres as a reference (Table 2.4) (New, 2006).

A condition of failed two consecutives platelet transfusions to achieve desirable increment is called platelet transfusion refractoriness. Platelet transfusion indications and management for patients with platelet transfusion refractoriness are commonly difficult to face. A review by Eldad Hod and Joseph Schwartz structured an algorithm for managing platelet transfusion refractoriness that required identical ABO group and fresh component with cross-matching principal, as displayed in Figure 2.1 (Hod and Schwartz, 2008).

Table 2.3: Indication for platelet transfusion.

Clinical indication	Cut-Off values of platelet count
Hematological malignancies	<20 x 10^9/L is the safe limit unless: fever, bleeding, on antibiotics or coagulopathy
Procedures:	
1.Bone marrow aspiration and trephine biopsy	<20 x 10^9/L providing adequate surface pressure is applied
2.Lumbar puncture, epidural, OGDS and biopsy, indwelling lines, transbronchial biopsy, liver biopsy, laparatomy	<50 x 10^9/L
3. For operation at critical sites: eye and brain	<100 x 10^9/L
Massive transfusions:	
1.Acute bleeding	<50 x 10^9/L
2.Multiple trauma/ Central nervous system (CNS) injury	<100 x 10^9/L
DIC:	
1.Acute DIC	Frequent estimation of platelet count and coagulation screen should be done. Aim to maintain platelet count at 50> x 109/L
2. Chronic DIC/ Absence of bleeding	Platelet transfusion should not given
Coronary artery bypass graft/ ruptured abdominal aortic aneurysm:	
Pre-operative assessment of medication KIV delay surgery for pre-operative transfusion	Should be reserved for those with post-operative bleeding and surgical cause has been excluded.
Immune thrombocytopenia:	
1.Autoimmune thrombocytopenia	Only for life-threatening bleeding from Gastrointestinal tract / Genitourinary tract /CNS and other conditions with severe thrombocytopenia (<10 x 109/L)
2.Neonatal autoimmune thrombocytopenia	Transfuse compatible platelet ASAP, ideally HPA-1a negative, HPA-5b negative. Platelet prepared from mother should be irradiated and washed.
3.Post transfusion purpura	Platelet transfusion usually ineffective
Platelet transfusion disorders: Platelet transfusion only indicated if other measures fail to control the bleeding	
Platelet transfusion is contraindicated in: Thrombotic thrombocytopenic purpura, Heparin induced thrombocytopenia and Kasabach Meritt Syndrome.	

(Adapted from (PDN, 2007))

Table 2.4: Suggested platelet transfusion thresholds for neonates.

Transfusion of platelet	**Platelet threshold**
Consider in all neonates	$< 30 \times 10^9$/L
Consider if increased bleeding risk, for example: 1. < 1000 g and < 1 week of age 2. clinically unstable (e.g. labile blood pressure) 3. previous major bleeding (e.g. grade 3–4 intraventricular haemorrhage) 4. current minor bleeding (e.g. petechiae) 5. coagulopathy 6. planned surgery or exchange transfusion	$< 50 \times 10^9$/L

(Adapted from (New, 2006))

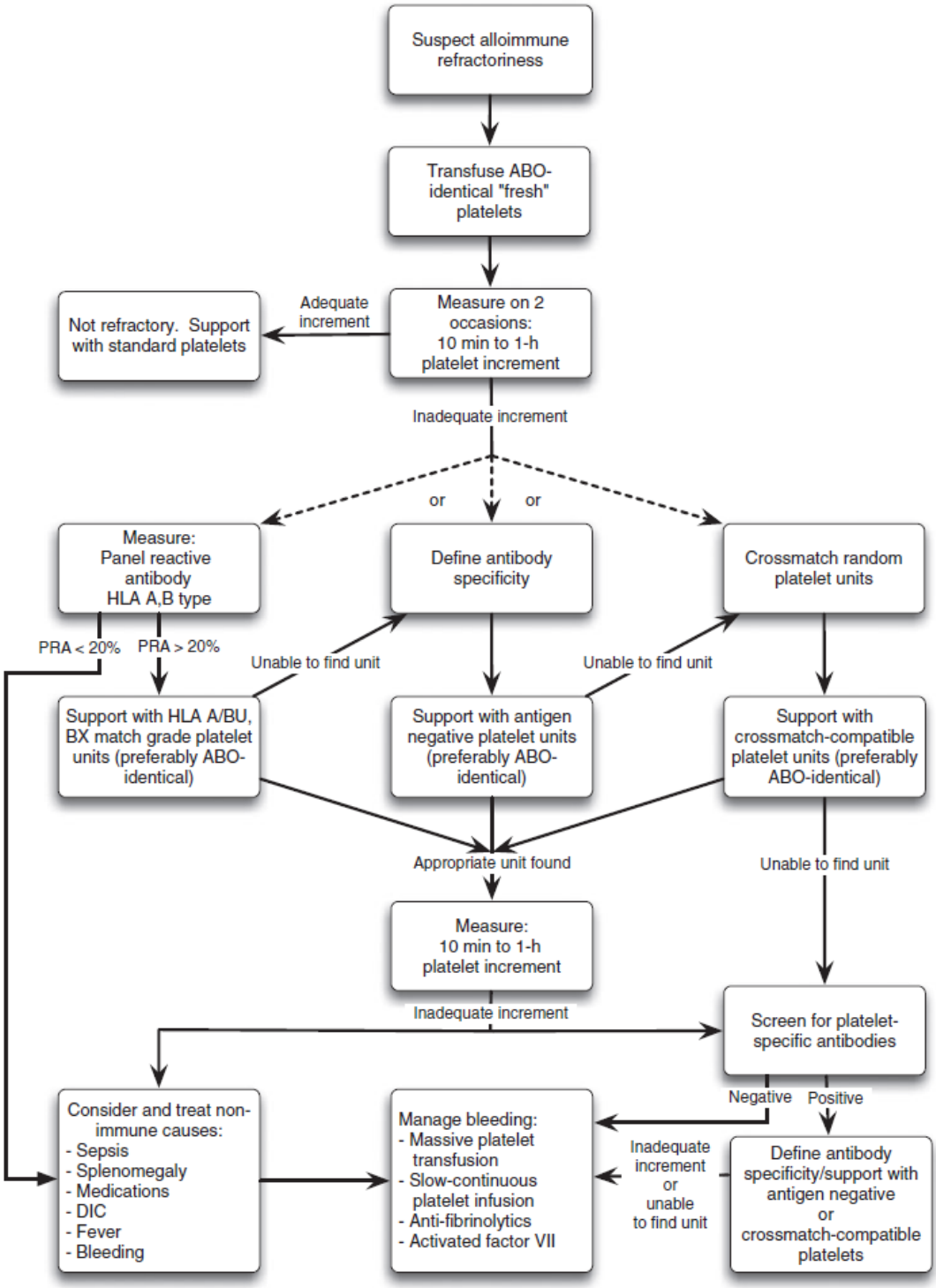

Figure 2.1: Approach for platelet transfusion refractoriness (Adapted from (Hod and Schwartz, 2008)).

2.3 Platelet selection for transfusion

2.3.1 ABO compatibility

It is well known, from earliest days that ABO blood groups were not essential consideration to elucidate normal response to a platelet transfusion. Hence, single donor and multiple donor platelet concentrates were given with no regard to the ABO status. In the recent practice, it is evident that ABO incompatibility can affect the adequacy of platelet transfusions, in addition, it can also have effects on the recipient wellbeing (Lozano and Cid, 2003).

Before we can understand the meaning of ABO compatibility, ABH group antigens on platelets need to be understood. Oligosaccharide structures is the main structure needed as a reside platform for glycosyltransferase to act, subsequentially on 2 types of precursor oligosaccharide chains called paraglobosides. The H gene encodes a fucosyltransferase that attaches fucose via an α(1-2) linkage to the terminal galactose of type 2 chains. The secretor (Se) gene encodes fucosyltransferase that act similarly on type 1 paragloboside chains. Individuals whose cells have no sugars added, other than fucose by the H transferase, are denoted blood group type O and express the H antigen. In group A, another glycosyltransferase adds an additional N-acetyl-galactosamine to the terminal galactose, whereas in B group individuals the B gene encodes a B enzyme that adds another galactose via α (l-2) bond (Lozano and Cid, 2003).

When people who have a certain blood type receive blood from someone with a different blood type, it may cause their immune system to react. This is called ABO incompatibility. Still, most blood bank centres use serology compatibility test which is mediated by antigen

antibody reaction only for packed red cells and not for platelet concentrates. Thus, the selection for platelet concentrates for transfusion rely more on ABO identical, best matched available recipient and donor platelet concentrate ABO group status (Shehata *et al.*, 2009). Furthermore, red blood cells contamination in the product is usually negligible (less than 2 mL) and compatibility testing is practically not required, but it is recommended to transfuse the donor plasma which is ABO-compatible with the recipient, especially neonatal transfusions (Denise M. Harmening, 2005).

On the other hand, guidelines of platelet transfusion by British Journal of Hematology recommended that platelet transfusion with identical ABO group is the best choice, because ABO non-identical platelet transfusion has been associated with poorer platelet count increment. Still, administration of ABO non-identical platelets is acceptable transfusion practice, in particular, when identical ABO platelet concentrates are in short supply, or when HLA-matched platelets are required and the best match is not ABO compatible (Haematology and Force, 2003).

Besides that, in the guidelines it is stated that group O platelets should only be used for group A, B and AB patients if they have been tested and labelled as negative for high-titre anti A and anti B. This becomes a concerns due to the possibility of hemolysis in transfusion of group O platelet concentrates to patients of other ABO groups (Haematology and Force, 2003). This statement was supported by a retrospective study which showed the occurrence of hemolysis due to ABO minor mismatched platelet transfusion, but the incidence was quite rare (Menis *et al.*, 2012).

It has been noted that transfusion of ABO-incompatible platelets may end up with intravascular hemolysis and it is not definitively known whether ABO matching of platelet

transfusion improves the outcomes. Furthermore, any intention to provide ABO-identical platelet can be troublesome due to the fact that most centres have limited inventories (Fung *et al.*, 2007) . A review article by Shehata et al had made definitions and descriptions of ABO-matched transfusions based on many researches (Table 2.5)(Shehata *et al.*, 2009).

Table 2.5: Definition of ABO-matched transfusion.

ABO matched groups	Description
ABO-identical	platelet transfusion as both the donor and the recipient being the same ABO type (e.g. Recipient A positive received platelet concentrate from donor A positive).
ABO-compatible	platelet transfusion when the donor platelets do not carry A or B antigen that are incompatible with the recipient's plasma (e.g. Recipient A positive received platelet concentrate from donor O positive).
ABO-incompatible	platelet transfusion when the donor platelets do carry A or B antigen that are incompatible with the recipient's plasma (e.g. Recipient A positive received platelet concentrate from donor B positive).
ABO-non-identical	platelet transfusion to reflect ABO-compatible and ABO-incompatible transfusion (e.g. Recipient A positive received platelet concentrate from donor B or AB or O positive).

(Adapted from (Shehata *et al.*, 2009))

The ABO compatibility can also be classified by its cross-matching component, as shown on Table 2.6 base on a review done by Joan Cid (Cid *et al.*, 2013).

Table 2.6: ABO mismatches classification

Recipient ABO type	ABO-major mismatched (recipient has isohemagglutinins against ABO antigens on donor platelet)	ABO-minor mismatched (donor has isohemagglutinins against ABO antigens on recipient red blood cell)
A	B, AB	B, O
B	A, AB	A, O
AB	none	A, B, O
O	A, B, AB	none

*Note that as group AB recipients lack anti-A and –B, a major mismatched platelet transfusion cannot occur, and as group O platelet lack A or B antigens, a minor mismatched transfusion cannot occur (Adapted from (Cid *et al.*, 2013)).

There are few strategies that can be used to reduce the risk of hemolysis, some centres wash or volume reduce the incompatible plasma from the platelet unit. Washing method is most effective removing plasma protein, however there are some drawbacks. It can potentially lead to decreased platelet function and platelet aggregation in vitro (Tynngård *et al.*, 2010; Veeraputhiran *et al.*, 2011). The other strategy that can be applied, only ABO identical platelets or ABO major mismatched platelets would be issued and if this component is low in stock then washing/volume reducing minor mismatched platelet might be considered to be use next (Shehata *et al.*, 2009).

2.3.2 Rhesus-D (RhD) compatibility

The Rhesus (Rh) blood group system is the second most important blood group system, after ABO. Until now, the Rh blood group system consists of 50 defined blood-group antigens, among which the five antigens D, C, c, E, and e are the most important. The commonly used terms Rh factor, Rh positive and Rh negative refer to the presence or absence of D antigen only, respectively (Landsteiner and Wiener, 1941). However, Rhesus antigens are not expressed on platelet surface. This makes rhesus compatibility for platelet transfusion not so important (Dunstan *et al.*, 1984).

Still, RhD-negative patients are best to be given RhD-negative platelet concentrates particularly to women who have not reached the menopause. If transfusion of RhD-positive platelets is unavoidable, anti-D should be given except for men or women without childbearing potential. The main concern for this selection of platelet concentrates is protection of RhD-

negative females at the childbearing age from developing alloimmunization by receiving RhD-positive red cells contaminant in platelet concentrates. If alloimmunization occurred in subsequent pregnancy, a hemolytic disease of newborn may complicate the RhD-negative mother (Haematology and Force, 2003).

2.3.3 Irradiated platelet concentrates

Any platelet concentrate which undergo gamma-emitting treatment is called irradiated platelets. This irradiated platelet concentrates are functionally maintained up to 5 days shelf life from collection date and show no clinical significant changes in platelet transfusion (PDN, 2015). The radiation given should be a minimum dose of 25Gy to consider it effective against donor lymphocytes (Murphy *et al.*, 1992).

All patients who have risk of transfusion-associated graft-versus-host disease are highly recommended to be transfused with irradiated platelets only (Haematology and Force, 2003). According to PDN guidelines there are few condition that require for irradiated platelet transfusion, as summarized in table 2.7(PDN, 2015).

Table 2.7: Indications for irradiated platelets.

1. In cases where blood is taken from related donors
2. For allogenic stem cell transplants
3. Infants under 4 months of age
4. Intrauterine and exchange transfusions
5. Immuno-compromised patients due to disease or its treatment ie: Severe Combined Immune Deficiency Syndrome(SCIDS), Common Variable Immune Deficiency(CVID), Malignancy Transplant recipients (bone marrow and solid organ), Hematology/Oncology patients

(Adapted from (PDN, 2015))

2.3.4 Cytomegalovirus-seronegative platelet concentrates

Cytomegalovirus (CMV) is a common virus that infects people of all ages. In the United States, nearly one in three children are already infected with CMV by age 5 years. Over half of adults by age 40 have been infected with CMV. Once CMV is in a person's body, it stays there for life and can reactivate. A person can also be re-infected with a different strain of the virus (CDC, 2016).

In the West, transfusion-transmitted CMV infection may cause significant morbidity and mortality in immunocompromised CMV-seronegative patients. This type of platelet concentrates is indicated for CMV seronegative pregnant women, intrauterine transfusions and CMV-seronegative allogeneic haemotopoietic stem cell transplant recipients; CMV-seronegative blood is also indicated for patients undergoing solid organ transplants, CMV-seronegative patients with conditions likely to require allogeneic haemotopoietic stem cell transplantation and CMV-seronegative patients with HIV infection (Haematology and Force, 2003).

In Malaysia however, mass screening for CMV as transfusion transmitted infection (TTI) is not beneficial as this virus is endemic in this region. A study based on serology survey in Hospital Universiti Kabangsaan Malaysia showed 91.8% of thalassemic patients had antibody to CMV (Jamal *et al.*, 1998). Meanwhile, in Kelantan the percentage of seropositivity in blood donors of CMV antibodies was even higher, about 97.6% (Ahmed *et al.*, 2006). Despite that, CNS involvement (as a parameter of severe CMV infection) is significantly higher in developed countries like UK (20%), and United States (20%) compared to Malaysia (10.5%) (Balasubramaniam *et al.*, 1994).

2.3.5 Leucodepleted/ leucoreduced platelet concentrates

A leucoreduced platelet concentrate is a component that most of its leucocytes had been removed, where leucocytes count is less than 1 x 10^6 (PDN, 2015). In achieving this leucoreduction, filtration is usually performed either in the blood bank prior to release of the platelets concentrate or at the bedside. The main indications to give a leucoreduced platelet concentrates are to prevent febrile non-hemolytic transfusion reactions, to prevent or delay the development of HLA antibodies and to reduce the risk of transmitting CMV(PDN, 2007).

2.3.6 Platelet transfusion for neonates

There is some additional specification for neonatal platelet transfusion, such as the components should be free of clinically significant irregular blood group antibodies, including high titre anti-A and anti-B, negative for CMV and should contain > 40 x 10^9 / L platelets. Meanwhile, for intrauterine transfusion the platelet concentrates should be hyperconcentrated platelet components (> 2000 x 10^9 / L) prepared from apheresis platelets with the aim of limiting the volume of the transfusion to the fetus, should contain < 0.0025 x 10^9/L leucocytes in each preparation, should be used within 24 hr after apheresis collection, negative for CMV, gamma irradiated and should contain > 120 x 10^9 / L platelets in 60 mL of plasma (Haematology and Force, 2003; New, 2006).

2.4 Monitoring response to platelet transfusion

Calculation of platelet recovery and corrected count increment

Every platelet transfusion should be monitored as its value can be uses as guide in future platelet transfusion therapy. However, there is no evidence that monitoring and action taken base on the result of responses to platelet transfusions decreases the incidence of bleeding event (Schiffer, 2001; Slichter, 2004) . In general, poor corrected count increment (CCI) following at least two fresh ABO compatible concentrates transfusion warrant the clinician to search for histocompatible platelet, because sometime patient who receives single platelet transfusion with poor increment will have satisfactory increment in second transfusion and if clinician use only single transfusion as a marker for refractoriness, this data is surely will miss leading (Schiffer, 2001).

In calculating the significant increment value of platelet transfusion, CCI and percentage platelet recovery (PPR) have been widely used and internationally accepted. Still, few studies showed the CCI equation had better value in comparing response of platelet transfusion among patients compared to PPR (Bishop *et al.*, 1992; McCullough *et al.*, 2004). Nevertheless, PPR is more superior in reflecting the immediate platelet viability, whereas assessing the platelet survival will depend on late platelet CCI estimated after 18-24 hours (hr) or inter-transfusion interval (Schiffer, 2001; Haematology and Force, 2003; Slichter, 2004; Rebulla, 2005; Blajchman *et al.*, 2008). Both equations require values of platelet level pre and post transfusion with correlation to patient body weight for PPR and body surface area for CCI (Haematology and Force, 2003; Brecher, 2005).

However, there were some contradicting findings for these equations. Hervig et al found that these formulas gave some inaccurate results due to bias and unstandardization in automated analyzers, while Davis et al illustrated both formulas may not give accurate results in obese patient (Davis *et al.*, 1999; Hervig *et al.*, 2004).

A successful platelet transfusion will be considered in stable patients if the PPR achieves about 67%, but generally accepts minimum of PPR and CCI to be concluded as successful transfusion are PPR > 30% at 1 hr post transfusion and >20% at 20-24hr , or a CCI of > 7.5 x 10^9/L at 1 hr and >4.5 x 10^9/L at 20-24hr, respectively (British Committee for Standards in Haematology, 2003).

The CCI (X 10^9/L) is calculated from the corrected count increment (PI), the body surface area of the patient in square metres (BSA) and the dose of platelet transfused (X 10^{11}((PD) [in general, 1 RDP= 0.6 x 10^{11} ; 1 apharesis platelet > 2.0 x 10^{11}] (Apelseth *et al.*, 2011)

$CCI = PI \times BSA \times PD^{-1}$

While the percentage platelet recovery (PPR) or post-transfusion platelet recovery is calculated from the platelet increment (10^9/L) (PI), the blood volume (BV) in litres and the platelet dose transfused (X 10^9) (PD). The blood volume estimate most commonly used in the calculation is 75 mL/Kg (McFarland, 2008).

$PPR (\%) = PI \times BV \times PD^{-1} \times 100$

The actual definition of platelet refractoriness varies, but one widely accepted definition (as applied in the Trial to Reduce Alloimmunization to Platelets) is two of 1 hr CCI values on consecutive days of less than 5000. Other definition for platelet refractoriness is a consecutive CCI less than 7500/μL measured 10–60 minutes after a transfusion, and less than 4500/μL measured 18–24 hr after a transfusion (Hendrickson and Roback, 2009).

2.5 Factors influencing post-transfusion platelet increment

2.5.1 Platelet transfusion factors

2.5.1(a) ABO compatibility platelet transfusion

There was two trial studies that showed 39 % and 61 % of ABO identical platelet transfusion had reduced the frequency of platelet refractoriness in patients with various hematologic malignancies, respectively (Carr *et al.*, 1990; Heal *et al.*, 1993). ABO-identical platelet transfusion was also associated with significant 27% higher of CCI compared to non-identical platelet. Furthermore, this study also concluded that ABO-identical platelet transfusions were associated with higher CCI than ABO compatible or ABO incompatible (7500 vs 4800 vs 3000/μL, respectively) (p-value< 0.05) (Heal *et al.*, 1993).

Besides that, among the patients receiving ABO identical platelets, only 18% became refractory to platelet transfusion during their first ten transfusion compared with 53% of the non-identical group (p-value<0.01) and the onset of refractoriness was delayed in recipients of ABO identical platelet (Heal *et al.*, 1993).

A study by Garratty demonstrated that hemolytic transfusion reaction in major mismatched platelet transfusion was not significant. The reason given was that, dilution effect of recipient plasma on donor antibodies or the antibodies were neutralized by endothelial based A and B antigens may be the possible causes. However, this study could not conclude that major mismatches may not influence the post- transfusion platelet increment (Garratty, 1998).

Nonetheless, it has been known for many years that, major mismatched platelet transfusion has poor post-transfusion platelet increment and usually has positive correlation with high risk bleeding outcome. Few small retrospective studies on hematology-oncology patients have reported significantly lower post-transfusion platelet increment after ABO major mismatched platelet transfusion compared to ABO matched and ABO minor mismatched platelet groups (Heal *et al.*, 1987; Slichter *et al.*, 2005).

2.5.1(b) Rhesus-D (RhD) status with post-transfusion platelet increment

The general term of RhD positive and negative refer to the presence or absence of the D antigen. For platelets however, their surface membrane do not express the Rhesus, Duffy, Kidd, Kell and Lutheran antigens. Thus, indirectly makes RhD compatibility insignificant for platelet transfusion. A study by Dunstan et al showed the insignificant association of RhD status of the patient or donor pertaining to post-transfusion platelet increment (Dunstan *et al.*, 1984). Yet, it had been regulated that every platelet concentrate need to be labelled with the RhD status as its can cause anti-D alloimmunization in RhD negative patients if those patients receive RhD positive platelet components (Haematology and Force, 2003)