COMPARISON OF PLATELET FUNCTION AND PLATELET PARAMETERS OF APHERESIS PLATELET USING PLATELET ADDITIVE SOLUTION AND WITHOUT PLATELET ADDITIVE SOLUTION

By

DR SUMAIYAH ADZAHAR

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT

OF THE REQUIREMENT FOR THE DEGREE OF MASTER

OF PATHOLOGY (HAEMATOLOGY)

SCHOOL OF MEDICAL SCIENCES

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LIST OF ABBREVIATIONS

ACD	Acid citrate dextrose
ATP	Adenosine triphosphate
ATRs	Allergic transfusion reactions
CPD	Citrate phosphate dextrose
CFC	Continuous flow centrifugation
FDA	Food and Drug Administration
FESEM	Field Emission Scanning Electron Microscopy
FNHTRs	Febrile nonhemolytic transfusion reactions
FBC	Full blood count
FBP	Full blood picture
IFC	Intermittent flow centrifugation
MPV	Mean platelet count
PAS	Platelet additive solution
PC	Platelet concentrate
PDW	Platelet distribution width
PRP	Platelet rich plasma
RBC	Red blood cell
RDP	Random donor platelet
UPT	Unit Perubatan Transfusi
VWF	Von Willebrand factor

ABSTRAK

PERBANDINGAN FUNGSI DAN PARAMETER PLATELET AFERESIS MENGGUNAKAN LARUTAN TAMBAHAN PLATELET DAN TANPA LARUTAN TAMBAHAN PLATELET

Pengenalan: Larutan tambahan platelet (PAS) merupakan media nutrien kristaloid yang digunakan untuk menggantikan dan mengurangkan kira-kira dua pertiga plasma dalam produk platelet. Ianya merupakan medium alternatif yang diperlukan untuk menjaga kualiti platelet dengan jangka hayat yang lebih panjang.

Objektif: Tujuan kajian ini adalah untuk membandingkan fungsi platelet dan parameter platelet aferesis yang disimpan dalam PAS sebagai kumpulan yang dirawat dan tanpa PAS (plasma) yang merupakan amalan biasa sebagai kumpulan kawalan.

Rekabentuk kajian dan kaedah: Satu kajian intervensi telah dijalankan dengan melibatkan penderma darah aferesis di Unit Perubatan Transfusi, Hospital USM dari Februari 2019 sehingga Jun 2019 dan sejumlah 20 orang penderma darah aferesis yang memenuhi kriteria masuk dan kriteria singkir telah dipilih. Platelet aferesis diambil dengan menggunakan pembahagi sel Amicus dan disimpan dalam PAS dan tanpa PAS selama tujuh hari. Parameter platelet termasuk kiraan platelet, purata isipadu platelet (MPV), taburan keluasan platelet (PDW), penilaian morfologi platelet menggunakan mikroskopi, ujian agregasi platelet dan pH telah diukur pada hari pertama sehingga hari ketujuh dalam medium PAS dan tanpa PAS. Analisis statistik yang digunakan untuk parameter ini adalah *Repeated Measure ANOVA*. Parameter lain seperti reaktiviti titer antibodi ABO hanya diukur pada hari pertama penyimpanan dan dianalisis menggunakan statistik *Wilcoxon Signed Rank*. Kultur bakteria dijalankan pada hari ketujuh

penyimpanan dan dianalisis menggunakan ujian *Chi Square*. Nilai *p* iaitu <0.05 dianggap signifikan dari segi statistik

Keputusan: Majoriti penderma aferesis adalah daripada penderma lelaki Melayu. Purata kiraan platelet di dalam platelet aferesis adalah 3 – 4 x10^{11} per unit. Terdapat perbezaan signifikan pada MPV (hari ke tujuh, *p*=0.004), platelet aggregasi dengan asid arakidonik (hari ke tujuh, *p*=0.001), kolagen (hari pertama, *p*=0.011) and epinefrin (hari pertama, *p*=0.005; hari ke tujuh, *p*=0.041), morphologi platelet (hari pertama dan ke tujuh, *p*=0.041), pH (hari ke pertama, *p*=0.014; hari ke tujuh, *p*=0.002) and titer ABO antibodi (anti-A, *p*=0.002; anti-B *p*=0.004) antara aferesis platelet didalam PAS dan tanpa PAS. Parameter yang lain adalah tidak signifikan secara statistik.

Kesimpulan: Platelet aferesis yang disimpan dalam PAS menunjukkan keputusan yang inferior pada kebanyakan parameter yang dikaji berbanding dengan yang disimpan tanpa PAS, manakala parameter titer ABO antibodi dalam PAS menunjukkan keputusan yang lebih superior daripada tanpa PAS. Morfologi dan fungsi platelet lebih berkesan disimpan dalam plasma (tanpa PAS) jika dibandingkan dengan disimpan di dalam PAS. Walaubagaimanapun, kajian ini perlu disahkan dengan menggunakan lebih banyak sampel yang melibatkan kajian in-vivo pada masa hadapan.

ABSTRACT

COMPARISON OF PLATELET FUNCTION AND PLATELET PARAMETERS OF APHERESIS PLATELETS USING PLATELET ADDITIVE SOLUTION AND WITHOUT PLATELET ADDITIVE SOLUTION

Introduction: Platelet additive solution (PAS) is a crystalloid nutrient media practically used to replace and reduce approximately two-thirds of plasma in platelet product. It is an alternative medium intended to maintain good quality of platelets with longer shelf life.

Objective: The aim of this study is to compare the platelet function and platelet parameters of apheresis platelets store in PAS as the treated group and without PAS (plasma) which is the standard practice as a control group.

Study design and methods: An interventional study was conducted involving apheresis blood donors in Unit Perubatan Transfusi, Hospital USM from February 2019 until June 2019, and a total of 20 apheresis blood donors that fulfilled the inclusion and exclusion criteria were selected. Apheresis platelets were collected using Amicus cell separator and were stored in PAS and without PAS for seven days. Platelet parameters including platelet count, mean platelet volume (MPV), platelet distribution width (PDW), platelet morphological assessment using light microscopy, platelet aggregation test and pH were measured on day one and day seven of storage in PAS and without PAS. The statistical analysis used for these parameters was repeated measures ANOVA. Other parameters such as ABO antibody titer was measured only on day one of storage and was analyzed using Wilcoxon Signed Ranks test. The bacterial cultures were carried out on day seven

of storage and analyzed using Chi-square test. A *p* value of <0.05 which was considered statistically significant.

Results:

Majority of the apheresis donor was Malay male. The average platelet count of apheresis platelets was between 3.0 – 4.0 x10^{11}/unit. There were significant differences in the MPV (day 7, *p*=0.004), platelet aggregation test with arachidonic acid (day 7, *p*=0.001), collagen (day 1, *p*=0.011) and epinephrine (day 1, *p*=0.005; day 7, *p*=0.041), platelet morphology (day 1 and day 7, *p*=0.041), pH (day 1, *p*=0.014; day 7, *p*=0.002) and ABO antibody titers (anti-A, *p*=0.002; anti-B *p*=0.004) between apheresis platelet in PAS and without PAS. Other platelet parameters were statistically not significant.

Conclusion: The apheresis platelets stored in PAS demonstrated inferior findings in most study parameters compared to without PAS, while the study of antibody titer in PAS showed superior results compared to without PAS. The platelet morphology and function were better maintained in plasma (without PAS) compared to in PAS. However, this study needs to be confirmed with more samples and include in-vivo study in the future.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Platelet transfusion is clinically indicated whether as part of treatment or as prophylaxis in patients with platelet-related disorders. Platelet concentrates (PC) are indicated therapeutically to treat bleeding manifestation in a patient who has thrombocytopenia or qualitative functional defects (inherited or acquired). PC may also be administered prophylactically to prevent bleeding when the platelet count is below acceptable limit such as before a surgical procedure (Wood *et al.*, 2016).

PC can be produced from whole blood or by apheresis collection procedure. Approximately 85% of platelet transfusions are collected by apheresis in the United States (US) because of the high yield and minimal red blood cell (RBC) contamination. In Malaysia, apheresis platelet supply is mainly available in referral hospitals throughout the country. Platelets are routinely stored in plasma for five days at 22 - 24^{0}C with continuous agitation. During the storage period, platelets undergo structural, functional and biochemical changes that are recognized as platelet storage lesions (Bashir *et al.*, 2014). Hence platelet storage changes are a serious issue in transfusion medicine as these natural alterations may affect the transfusion outcomes. Optimizing synthetic media might help to attenuate the platelet storage half-life, thereby facilitating extended storage.

Platelet additive solutions (PASs) were introduced in the 1980s and several reformulations have been done to improve its efficacy in the subsequent years. They are used for storage of PC as an alternative to donor plasma by helping to improve the platelet quality and function as well as increasing their shelf lives. PAS volume replacement by

approximately two-thirds of donor plasma volume was suggested for optimal storage of platelets from previous studies (van der Meer and de Korte, 2018).

PAS is an electrolyte solution which contains acetate, citrate, phosphate, sodium, potassium, magnesium and glucose. Acetate helps in lowering glucose conversion to lactic acid, thus preventing low pH in the suspensory medium. The oxidation of acetate produces bicarbonates which have buffering property. The presence of potassium and magnesium in PAS solution is also believed to lower the rate of spontaneous platelet activation during the storage period (van der Meer and de Korte, 2018).

In conventional practice, the short half-life of PC stored in native plasma makes the inventory management of this blood component difficult (Pagano *et al.*, 2019). Hence an alternative to the plasma medium is desired for maintaining good quality platelet with longer shelf life. Presently, PAS has become a beneficial alternative to standard plasma as a suspending medium for platelet storage up to day seven (van der Meer, 2016). The use of PAS is gradually adopted in several countries and becoming a well-received alternative medium for platelet storage. However, more data are required to support the benefits of PAS in transfusion medicine. Practically, many of these studies were done in cold–climate countries and PAS medium may be affected since platelet is exposed invariably to a higher temperature during processing and blood collection procedure.

The aims of this study were to compare the platelet function and platelet parameters of apheresis platelet stored for seven days in PAS and without PAS (plasma) as the suspending medium. The study parameters included were platelet indices (platelet counts, MPV and PDW), platelet morphology, platelet function test, pH, ABO antibody titer and detection of bacterial contamination. In Malaysia, use of PAS as suspending medium for platelets is not practiced routinely due to various reasons including cost and

impact of platelet supply and demand. In addition, no extensive quality study was done on in-vitro platelet parameters when stored in platelet preservative (PAS) medium in local and regional transfusion centers.

CHAPTER 2

LITERATURE REVIEW

2.1 Platelets

In hemostasis and thrombosis, platelets are small cells that play an essential role. They are involved in diseases with a direct primary disorder of platelet number and function or indirectly from thrombosis such as stroke, coronary heart disease and diabetes (Linden, 2013). Normally, platelets circulate in the bloodstream in an inactive form, but will undergo rapid changes when they become activated. The platelet count in humans usually ranges from 150 – 400 $x10^9$ /L. Low platelet count is associated with an increased risk of bleeding during surgery or major trauma.

2.1.1 Platelet structure and formation

Platelets are produced in bone marrow by fragmentation of the cytoplasm of megakaryocytes. Thrombopoietin (TPO) is the primary regulator for platelet formation, and 95% is produced by the liver. Platelets circulate in the blood in a small, granular, discoid shape with dimensions in the human of approximately 3.0 x 5.0 um and a mean volume of 7 -11 fL. This shape and size enable platelets to be marginated by red blood cells to the edge of the vessel, placing them in contact with the vascular endothelial cell. In the presence of vessel wall damage, they will undergo rapid adhesion, activation and aggregation to form a hemostatic plug, thus preventing blood loss. For the initiation of the coagulation cascade, they also provide a phospholipid surface. Platelets lack a nucleus and the lifespan is short which is between 5 to 7 days following formation and separation from the megakaryocytes. They are removed from the vessel by neutrophils and

macrophages. Subsequently, they are destroyed in the spleen at the end of their life (Holinstat, 2017).

Platelets contain three types of granules that release their contents when they become activated (Figure 2.1). The most common type is an alpha granule. It is about 200-500nm in diameter and contains proteins that support platelet adherence. These proteins include fibrinogen, von Willebrand factor (VWF) and vitronectin that may contribute to stabilization and thrombus formation. Alpha granules also contain a variety of adhesion molecules, coagulation and fibrinolysis proteins, immunologic molecules, growth factors and chemokines (Linden, 2013). Dense granules contain adenosine triphosphate (ATP), adenosine diphosphate (ADP), calcium and serotonin.

These factors contribute to platelet recruitment and thrombus formation following injury in the vessel. The third type of granules is the lysosomal granules that contain acid hydrolase, cathepsins and lysosomal membrane proteins. These granules play an important role in degrading protein (Holinstat, 2017).

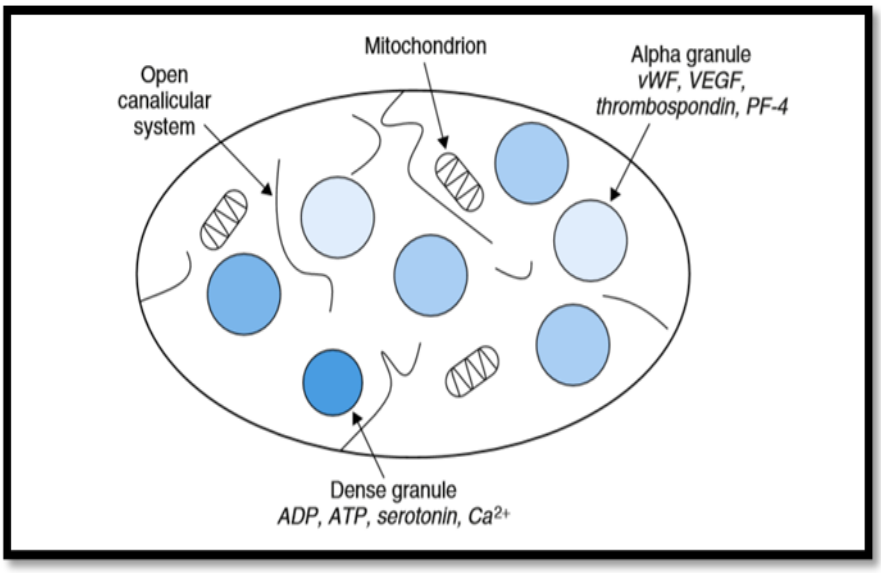

Figure 2.1 An overview of the platelet structure. Adopted from (Reyes Gil, 2019)

2.1.2 Role of platelets in hemostasis

Hemostasis is a physiological process that stops bleeding at the site of injury. The primary wave of hemostasis is platelet accumulation, followed by a secondary wave that is mediated by the blood coagulation pathway. Platelets play a critical role in sequential events during platelet accumulation. They are also involved in cell-based thrombin generation that amplifies the blood coagulation pathway. Thus, platelets contribute to both primary and secondary waves of hemostasis. Platelet encourages primary hemostasis via three major processes; activation, adhesion and aggregation (Hou *et al.*, 2015).

Platelet adhesion to the injured vessel wall is generally supported by the interaction between membrane receptors and plasma proteins. The platelet membrane receptors consist of glycoprotein receptors embedded in the phospholipid bilayer. These receptors also include tyrosine kinase receptors, integrins, leucine-rich receptors, G-protein coupled transmembrane receptors, selectins and immunoglobulin domain receptors. These proteins mediate interaction within cell platelet and platelets substrates. The first event that occurs upon hemostasis is the rolling and adherence of the platelets to the exposed subendothelium. Platelet adhesion is mediated by VWF that binds to Gp1b-IX in the platelet membrane. VWF is a blood glycoprotein that serves as an adhesive protein that could bind to other proteins, especially factor VII at the injured site (Periayah *et al.*, 2017).

Adhered platelets become activated and undergo degranulation and release the alpha and dense granules that contain serotonin, platelet-activating factors and ADP (Figure 2.2). ADP is an agonist that plays an important function in normal hemostasis and thrombosis. Activated platelets change their shape into pseudopodal form and activate the collagen receptors on their surface membrane known as GpIIb/IIIa to undergo release

reactions. Besides, GpIIb/IIIa integrins and P-Selectin move from the alpha granule membrane to the platelet membrane to support platelet aggregation. Platelets also tend to produce thromboxane (A29TXA2) in addition to vasoconstriction and platelet aggregation (Periayah *et al.*, 2017).

In addition, each activated platelet extends their pseudopods, clumping, and become aggregated. Subsequently, these activations are further continued by the production of thrombin via hemostasis mechanism. Platelet aggregation promotes a primary platelet plug. Eventually, the platelet plug becomes stabilized by the formation of fibrin.

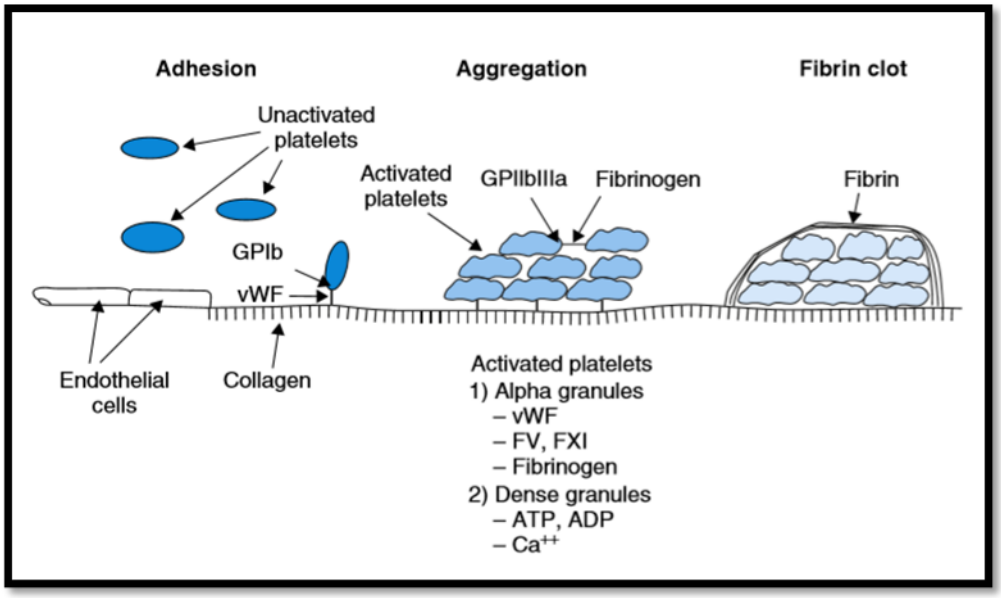

Figure 2.2 Overview of platelet-vessel wall interaction. Adopted from (Reyes Gil, 2019)

2.2 Platelet transfusion

Platelet transfusions are indicated as prophylaxis to prevent bleeding or for the treatment of bleeding. Patients with hematological disease are the largest group of patients that receive up to 67% of PC. The decision to transfuse platelets should be based on clinical assessment, taking into account clinical risk factors for bleeding and the extent and site of bleeding, as well as the platelet count. Several diseases including infectious and noninfectious can be transmitted via blood transfusion and its component. Therefore, the decision for platelet transfusion should take this factor into consideration. Several factors that can affect the outcome of platelet transfusion include patient, donor and platelet products (collection, preparation and storage condition) (Wood *et al.*, 2016).

2.2.1 Indications of platelet transfusion

2.2.1(a) Prophylactic platelet transfusion

The common practice for prophylactic platelet transfusion is to transfuse platelets when the platelet counts reach a transfusion trigger threshold in asymptomatic thrombocytopenic patient. Patient's platelet count determines the threshold with consideration of several factors such as underlying disease, presence of sepsis, medications and coagulation status. The British Committee for Standard in Hematology (BCSH) has recommended a platelet count of 10,000 /u L as transfusion threshold and a higher threshold may be used when bleeding, fever or sepsis are present.

Prophylactic platelet transfusion is also indicated in preparation for invasive procedure or surgery. Invasive procedures such as insertion of a central venous catheter, transbronchial biopsy, gastrointestinal endoscopy and biopsy can be performed with the platelet count higher than 40,000 to 50,000/ul. For procedures involving critical lesions

such as neurosurgery or ophthalmic surgery involving the posterior segments of the eye, maintenance of platelet count of more than 100,000/ul should be considered (Thiagarajan and Afshar-Kharghan, 2013).

2.2.1(b) Therapeutic platelet transfusion

There are few evidences for the effectiveness of therapeutic platelet transfusion or the optimal dose when a patient with thrombocytopenia is actively bleeding. According to BCSH guidelines, platelet transfusion should be considered in severe bleeding to maintain the platelet count above 50,000/ul. The platelet count above 100,000/ul should be maintained in patients with multiple trauma, traumatic brain injury or spontaneous intracerebral hemorrhage. However, in patients with bleeding that is not considered as life-threatening, platelet transfusion is considered if the platelet count is below 30,000/ul (Estcourt *et al.*, 2017).

Table 2.1 Guide for the use of platelet transfusion adapted from the Handbook on Clinical Use of Blood, National Blood Center, 2020.

Clinical indications	Cut-off values of platelet count
Hematological malignancies	>10 x 10^9/L is the safe limit unless: fever, bleeding, on antibiotic or coagulopathy.
Procedures:	
Bone marrow aspiration and trephine	>20 x10^9/L provided adequate surface pressure is applied.
Lumbar puncture, OGDS & biopsy, indwelling lines, transbronchial biopsy, liver biopsy, laparotomy	Platelet count should be raised to at least 50 x 10^9/L.
For operation at critical sites: eye & brain, epidural	Platelet count should be raised up to at least 100 x 10^9/L.

Table 2.1 continued

Clinical indications	Cut-off values of platelet count
Massive transfusion:	
Acute bleeding	Platelet count should be raised up to at least 50 $x10^9$/L.
Multiple trauma / CNS injury	Higher target level of 100 x 10^9/L.
Disseminated intravascular coagulation:	
Acute disseminated intravascular coagulopathy (DIC)	Frequent estimation of platelet count and coagulation screening should be done. Aim to maintain platelet count at >50 $x10^9$/L.
Chronic DIC / Absence of bleeding	Platelet transfusion should not be given.
Immune thrombocytopenia:	
Autoimmune thrombocytopenia	Only for life-threatening bleeding from gastrointestinal, genitourinary, central nervous system and other conditions with severe thrombocytopenia (<10 $x10^9$/L)
Neonatal autoimmune thrombocytopenia	Transfuse compatible platelet as soon as possible: HPA antigen negative. Platelet prepared from mother should be irradiated and washed.
Post-transfusion purpura	Platelet transfusion usually ineffective. May be used in acute phase e.g.: operation.
Platelet function disorder	
Platelet transfusion only indicated if other measures fail to control the bleeding.	

2.2.2 Contraindications of platelet transfusion

Not all patients with thrombocytopenia is indicated for platelet transfusion. In fact, in a few cases of thrombocytopenia, platelet transfusion is contraindicated. Generally, a patient with thrombotic thrombocytopenic purpura (TTP) or heparin-induced thrombocytopenia (HIT) will have a contraindication of platelet transfusion, except there is life-threatening bleeding. This is because evidently, platelet transfusion can precipitate further thrombotic events. In addition, a recent randomized controlled trial study showed that platelet transfusion had been associated with higher mortality in patients with acute, spontaneous intracerebral hemorrhage on antiplatelet therapies. Thus, platelet transfusion may also be contraindicated in this setting (Cushing and DeSimone, 2019).

2.3 Platelet concentrate preparation

PC can be produced from whole blood collections or by apheresis. Approximately 85% of platelet transfusions are collected by apheresis in the United States (US) because of the high yield and minimal RBC contamination. PC prepared from whole blood are referred to as random donor platelets (RDP) and when produced by apheresis method, it is called apheresis platelets. RDP should contain at least 5.5 x 10^{10} platelets, sufficient plasma (approximately 40 to 70 ml) to yield a pH of greater than or equal to 6.2, stored with continuous agitation at 20 - 24°C and have a shelf life of 5 days. Apheresis platelets should contain at least 3 x 10^{11} platelets (the therapeutic equivalent of four to six RDP) in approximately 300 ml of plasma, stored at 22°C- 24°C with continuous agitation and have a shelf life of 5 days (Harmening, 2012).

2.3.1 Platelet concentrate preparation from whole blood donation

Whole blood used to prepare platelet concentrates must be drawn by a single non-traumatic venipuncture. Then, it will be collected in a bag containing anticoagulant preservative solution (citrate phosphate dextrose (CPD)). The whole blood unit undergoes first centrifugation (soft spin) after which platelet-rich plasma (PRP) is produced and RBCs remain. Then, followed by a second centrifugation (hard spin) which separates the platelets from the plasma, the supernatant is transferred to another container leaving approximately 40 to 70 ml of plasma with the platelet suspension.

The platelet products must be pooled to make a sufficient adult dose and test negative for bacterial contamination prior to release. The Food and Drug Administration (FDA) has approved a system for pre-pooling, leukoreduction and bacterial testing using a culture-based system and these platelets are known as 'pre-storage pooled platelets'. About 3-6 units of platelets may be pooled using this system to achieve an FDA approved of 2.2-5.8 x 10^{11}. These pooled platelets also contain plasma from each donor and thus considered a risk for transfusion reaction (an example is transfusion-related acute lung injury (TRALI)) (Beth *et al.*, 2013).

2.3.2 Platelet concentrate preparation by apheresis donation

Apheresis is defined as "taking away", which was derived from an ancient Greek word. It refers to a process whereby whole blood is removed from the body of a donor and passed through an apheresis machine blood separator that separates specific blood components and returns the remainder of the components into donor's circulation.

This procedure has represented major advancement in blood component collection in which it increases the ability to produce optimal components for patients and prevents

wastage. Apheresis can be performed on a donor to collect a specific blood component. The proses of removing plasma are called plasmapheresis. Apheresis technology can also collect platelets (plateletpheresis), red blood cells (erythrocytapheresis) or leucocytes (leucopheresis) using similar method (Harmening, 2012).

Apheresis is currently performed using automated technology, and separation is done by centrifugation. Apheresis instruments have a computerized control panel, allowing the operator to select the desired component to be collected. Currently, available machines use disposable equipment, which includes sterile tubing tube sets, bags and a collection chamber unique to the machine. The amount of time for a particular procedure can range from 45 to 120 minutes. The most commonly used instruments exert one of two methods of centrifugation; intermittent flow centrifugation (IFC) and continuous flow centrifugation (CFC) (Harmening, 2012).

In IFC procedure, the blood is processed in cycles, hence the term intermittent. Whole blood is drawn with the assistance of a pump. Anticoagulant is mixed with the blood as it is pumped into a centrifuge bowl through the inlet port to prevent the blood from clotting. The bowl rotates at a fixed speed to separate the components according to their specific gravities. The RBCs are packed against the outer rim of the bowl, followed by WBCs, platelets and plasma. Once separated, the pump is reversed and the desired components are pumped through the outlet port into a collection bag. The undesired components are pumped into a reinfusion bag and returned to the donor, constituting one cycle (Harmening, 2012).

The cycles are repeated until the desired product is obtained; for example, a plateletpheresis procedure takes six to eight cycles to collect a therapeutic adult dose. The IFC procedure can be performed with only one venipuncture (blood is drawn and

reinfused through the same needle). This is also known as a single needle procedure. An example of an apheresis machine employed this method is Haemonetics MCS Plus LN9000 (Harmening, 2012).

As compared to IFC, the processes of blood withdrawal, processing and reinfusion are performed simultaneously in an ongoing manner by the CFC procedure. Since blood is drawn and returned continuously during the procedure, two venipuncture sites are required. Blood is drawn from the first venipuncture site with the help of a pump, mixed with an anticoagulant and collected in a chamber (Harmening, 2012).

The separation of the components is performed by centrifugation, and the specific component is diverted and retained in a collection bag. The remainder of the blood is reinfused to the donor via a second venipuncture site. Examples of the machines carrying out this concept are Baxter/Fenwal CS-3000 Plus and the Amicus, the Caridian BCT COBE Spectra and Spectra Optia and the Fresenius AS-104 (Harmening, 2012). In this study, the preparation of PC by apheresis technique was preferred due to a high yield of platelet and volume.

2.4 Platelet additive solutions

PASs are electrolyte solutions that are used for platelet storage. They were developed in the 1980s and had undergone many improvements and modifications over the following years. In general, various types of PASs (e.g PAS-I, PAS-II and PAS-III) can be used for apheresis and buffy coat platelets. Usually, the storage medium is composed of a mixture of plasma (generally 20 – 40%) and PAS (60 - 80%). PAS contains acetate, citrate, phosphate, sodium, potassium, magnesium and glucose. The

approximate formulations of several PASs are shown in Table 2.2. In this study, we use PAS – III (Intersol, Fresenius Kabi, Germany) to replace the plasma.

Table 2.2 Approximate Formulations of PAS (Hiba and Joseph, 2012)

Chemical	PAS-I Plasmalyte	PAS-II T - Sol	PAS-III Intersol	PAS-IIIM SSP	Composol PAS - G	M- Sol
NaCl (mM)	90	116	77	69	90	110
NaAcetate (mM)	27	30	30	30	27	15
NaCitrate (mM)	0	10	10	10	11	10
KCl (mM)	5	0	0	5	5	5
$MgCl_2$ (mM)	3	0	0	1.5	1.5	3
Phosphate (mM)	0	0	26	26	0	4
Na Gluconate (mM)	23	0	0	0	23	0
Glucose (mM)	0	0	0	0	0	30
$NaHC0_3$ (mM)	0	0	0	0	0	12

2.4.1 Advantages of platelet additive solutions

PAS contains some ingredients to support the platelet storage condition. Acetate is the key element for this reason. It is negatively charged and has to oxidize to be neutral in order to enter the mitochondria. While in oxidation state, acetate derives a hydrogen ion from its environment, thus increasing the pH level. Platelets switch their metabolism from oxidizing glucose as substrate (which can produces more lactic acid causing the pH to be lower and subsequently causing the platelet storage lesion) to oxidizing a mixture of acetate and glucose that can further reduce the production of lactic acid (van der Meer and de Korte, 2018).

A significant additional effect of acetate is its ability to maintain stable pH levels during the storage of platelets by the formation of bicarbonate. The oxidation of acetate in cells is preceded by its conversion to the acid form of the compound, i.e., acetic acid. The hydrogen ion that is essential for the conversion can be derived from lactic acid. Therefore, the production of hydrogen ion by glycolysis will be balanced by the removal of hydrogen ions through the oxidation of acetate (Gulliksson, 2000).

Magnesium and potassium are present in some PAS. Magnesium is considered as it reduces the platelet activation by inhibiting exposure of P-selectin, therefore decreasing the binding of fibrinogen to ADP-activated platelets and will significantly decrease ADP-induces platelet aggregation. A study by De Wild- Eggen *et al* demonstrated the beneficial effect of potassium and magnesium on platelet activation. They used PAS II and additional potassium and magnesium as the basis for platelet storage resulting in a pH on Day 7 of 7.15 versus 6.94 for PAS II alone ($p < 0.05$). A notable difference was found on platelet activation measured as CD62P expression, which was 23 ± 6% for platelets in PAS II with potassium and magnesium versus 50 ± 8% for platelets stored in PAS II alone ($p < 0.001$). Therefore, they conclude that the addition of potassium and magnesium inhibits CD62P expression and allows the storage period of platelets up to seven days (van der Meer, 2007).

Transfusion reactions to platelets can range from mild urticarial response to severe life-threatening events. The majority of cases are classified as either allergic transfusion reactions (ATRs) or febrile nonhemolytic transfusion reactions (FNHTRs). The cause for these reactions is due to the presence of plasma in which units are stored. ATRs are usually Type I hypersensitivity reactions that result from allergens in the donor plasma interacting with preformed immunoglobulin E antibodies in the recipient while FNHTRs are thought to be due to cytokines released by WBCs into the plasma (Cohn *et al.*, 2014).

Acute hemolytic transfusion reactions (HTRs) occur typically with transfusion of high titer antibody. Platelets express ABO antigens on their surface, and they are suspended in original donor plasma. For example, group O platelet donor would have no A or B antigen expression on his or her platelets, but there are high titer of anti-A, anti-B and anti-A, B antibodies in the plasma that has the potential to cause hemolysis of the red blood cells of non-group O recipient, thus implicating in HTRs (Kavallierou, 2015).

Reducing the plasma in the platelet units has been shown to reduce these types of transfusion reactions. Previously conventional techniques such as volume reduction and washing had been used to overcome this problem. However, these techniques are labor-intensive manipulations and can also compromise the quality of the product. Hence, using PAS is an alternative method for plasma reduction because much of the plasma can be replaced by it (Cohn *et al.*, 2014).

2.4.2 Disadvantages of platelet additive solution

There are some disadvantages associated with the use of PAS. For example, in the buffy coat method, the centrifugation of the buffy coat pool is more difficult due to the lower viscosity of PAS, and thus the platelet yield is generally 10-15% lower compared to plasma. Some studies also showed that some PASs exhibited a shorter storage time as well as a lower increment in platelet count compared to plasma (van der Meer, 2007). Besides, PAS platelets are more expensive than the average cost of without PAS platelets.

2.5 Parameters for in-vitro platelet assessment

2.5.1 Platelet indices

The actual number of platelets transfused should meet pre-established acceptable ranges since platelet transfusions are given as prophylaxis or for therapeutic purposes. In Malaysia, guidelines set by National Blood Center (NBC) define quality control requirement for platelet count in random platelet is >60 $x10^9$ platelets per unit and in apheresis platelets, platelet count must be >200 $x10^9$ per unit (Afifah *et al.*, 2016). As a result of platelet storage lesions that invariably occur, monitoring of platelet levels is essential to ensure that an adequate amount of platelets is transfused.

Platelet can be counted in whole blood using the same technique of electrical or electro-optical detection as they are used for counting red cells. An optical fluoresce platelet count has been introduced on some Sysmex analyzers, in addition to the traditional impedance count. A dye is used to stain the platelet membranes and granules. The fluorescent staining of the platelets allows the exclusion of non-platelet particles from the count. As part of platelet count analysis, the MPV and PDW will also be recorded. PDW indicates variation in platelet size which is also known as platelet anisocytosis. Higher PDW values reflect a larger range of platelet size, which may result from increased activation, destruction and consumption of platelet. MPV is a machine-calculated measurement of the average size of platelets found in the blood and the average of MPV is around 5- 8 fL. (Bain *et al.*, 2017).

2.5.2 Platelet function test

A platelet function test is indicated to help in the diagnosis of patients with bleeding disorders. Normal platelet function depends on extracellular Ca^{2+} and Mg^{2+} concentrations and the choice of anticoagulant in which the most preferred is citrated blood. Platelet function tests can be divided into six main groups; adhesion tests, aggregation tests, assessment of granular content, assessment of release reaction, investigation of prostaglandin pathways and tests of platelet coagulant activity (Key *et al.,* 2017). In this study, we used platelet aggregation test based on optical method to assess the platelet function of apheresis platelets in PAS and without PAS solution.

Platelet aggregation test

Light transmission aggregometry (LTA) is the gold standard for platelet function assessment. The downside to LTA is that it is time consuming and labor intensive. Nowadays, there is great demand for more convenient way to test platelet function. Hence an automated platelet aggregation method has been developed on a routine coagulation analyzer (Sysmex CS-2100, Kobe Japan). The technology is also based on the principle that as platelet aggregate in response to the addition of an exogenous platelet agonist, the sample becomes more transparent and thus, more light will pass through it. Five aggregating agents including Adenosine 5-diphosphate (ADP), collagen, ristocetin sulfate, arachidonic acid, and adrenaline are used to induce platelet aggregation to assess maximum aggregation (Ling *et al.,* 2017).

A. Principle

The light absorbance of platelet-rich plasma (PRP) decreases as platelets aggregate. The addition of different dosages of agonists triggers platelet activation, shape

change, primary and secondary aggregation events. The amount and rate of the fall is depends on platelet reactivity to the added agonist. Other variables such as temperature, platelet count and mixing speed must be controlled. The absorbance changes are monitored on a chart recorder (Bain *et al.*, 2017).

B. Interpretation

ADP agonist

ADP with low concentration of 0.5 to 2.5 µmol/L causes primary or reversible aggregation. ADP binds to a membrane receptor and releases Ca2+ ion. Reversible complex with extracellular fibrinogen forms, and subsequently, the platelets undergo a shape change reflected by a slight increase in absorbance. Reversible aggregation tends to occur as the bound fibrinogen adds to the cell-to-cell contact. In the presence of higher concentrations of ADP, an irreversible secondary wave aggregation is associated with the release of dense and α-granules. This will result in the activation of the arachidonic pathway (Bain *et al.*, 2017).

Collagen agonist

The aggregation response to collagen is preceded by a short lag phase. The duration of the lag phase lasts between 10 and 60s and it is inversely proportional to the concentration of collagen used. This phase is succeeded by a single wave of aggregation resulting from the activation of the arachidonic acid pathway and the release of the granules. In the presence of higher doses of collagen, >2 µg /ml causes a sudden increase in intraplatelet calcium concentration. Thus, it may bring out the release reaction without activating the prostaglandin pathway. This collagen response should always be measured using 1ug/ml and 4µg/ml concentrations (Bain *et al.*, 2017).

Ristocetin agonist

Ristocetin mainly reacts with VWF and the membrane receptor to induce platelet clumping. It also does not activate any of the three aggregation pathways and also does not cause granule release. Therefore, the response is assessed on the basis of the angle of the initial slope. The platelet response to 1.2 mg/ml is studied. High concentration above 1.4 mg/ml may cause nonspecific platelet agglutination as a result of the interaction between ristocetin and fibrinogen and protein precipitation (Bain *et al.*, 2017).

Arachidonic acid agonist

The function of arachidonic acid is to induce TXA2 generation and granule release even if there is a defect of agonist binding to the surface membrane or of the phospholipid-induced release of endogenous arachidonate. In the absence or inhibition of cyclooxygenase (e.g., aspirin effect), arachidonic acid will not produce normal aggregation (Bain *et al.*, 2017).

Epinephrine agonist

The platelet aggregation response to epinephrine resembles the ADP response, but there is no shape change that precedes aggregation. Such a reaction is obtained with the concentration of 2-10 µmol/ml (Bain *et al.*, 2017).

2.5.4 Platelet morphology

Platelet morphology can be assessed by light microscopy and electron microscopy. Under light microscopy using Romanowsky stains, a normal platelet has a discoid lentiform shape and a granular basophilic appearance and the size is about 1 to 3 µm in diameter. A small number of larger platelets measuring to about 5 to 7 µm can also

be seen. A well-stained platelet should be purple or purplish pink in color. Detailed analysis of the cytoplasmic organelles such as granules, mitochondria, dense bodies and so-called open canalicular system of platelets can be evaluated by electron microscopy (Moreno and Menke, 2002).

2.5.5 pH of apheresis platelets

The pH of the apheresis platelets product and random platelet concentrates must be above >6.4, as required by National Blood Center Malaysia (NBC) guideline. Apheresis platelet products must be in optimum pH to ensure the quality of the product. The pH of less than 6.0 or more than 7.4 could render the disc-shaped platelet to sphere formation, resulting in a marked loss of recovery in-vivo upon transfusion (Chandra *et al.*, 2011).

2.5.6 ABO antibody titer

Performing an antibody titration can help determine antibody concentration levels. Twofold serial dilutions of serum containing anti-A or anti-B antibody are prepared and tested against the suspension of RBCs that possess the target antigen. The titer level is the reciprocal of the greatest dilution in which agglutination of 1+ or greater is observed. Careful preparation is required when performing the antibody titer. Contamination from a tube with higher antibody concentration can lead to false elevated titer level results. To avoid this problem, changing pipette tips between each tube while preparing the dilutions is suggested (Harmening, 2012).

2.5.7 Bacterial culture

The absence of bacterial contamination in PC shall benefit the prolongation of the shelf-life of platelets. Platelets are particularly susceptible to bacterial growth due to storage temperature at 22°C. An organism that was introduced during phlebotomy, bacteria from the environment, or during transient donor bacteremia may proliferate and can cause severe clinical adverse events in the recipient.

CHAPTER 3

OBJECTIVES

3.1 General objectives

To evaluate the platelet function and platelet parameters of apheresis platelets stored in PAS medium and without PAS (plasma).

3.2 Specific objectives

1. To compare the level of platelet indices, platelet function test, platelet morphology and pH of apheresis platelets between PAS and without PAS on day 1 and day 7 of storage.
2. To compare the distribution of ABO antibody titer of apheresis platelets in PAS and without PAS medium on day 1 of storage.
3. To compare the proportion of bacterial contamination of apheresis platelets in PAS and without PAS medium on day 7 of storage.

3.3 Research hypotheses

1. There are significant differences in the level of platelet indices, platelet function test, platelet morphology and pH of apheresis platelets between PAS and without PAS on day 1 and day 7 of storage.
2. There is a significant difference in the distribution of ABO antibody titer in PAS and without PAS medium on day 1 of storage.
3. There is significant difference in the proportion of bacterial contamination in PAS and without PAS medium on day 7 of storage.