# Evaluation of the Provision of Crossmatch Compatible Platelets for Haematology Patients who have Acquired Non-Immune Refractoriness

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2022

# Evaluation of the Provision of Crossmatch Compatible Platelets for Haematology Patients who have Acquired Non-Immune Refractoriness

A thesis submitted to the University of Manchester for the degree of Doctor of Clinical Sciences in the Faculty of Biology, Medicine, and Health

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2022

# Abstract

Platelet demand is unpredictable and will relate to case mix, programmes of treatment requiring intensive platelet support and health service activity. Other relevant factors include the impact of clinical practice guidelines which have the potential when implemented to reduce the increase in demand or supress the clinical application of platelet component transfusion (Morris, K. 2018). Although platelet transfusions are vital to the ongoing management of patients with haematological malignancies, many patients become refractory to platelet transfusion. Refractoriness is defined as having a poor response (immediate or 24-hour increment post platelet transfusion of $<10 \times 10^9$/L) to random donor platelets on two or more occasions. Refractoriness can be due to either immune or non-immune mechanisms. Most platelet refractory cases are thought to be non-immune with the minority due to anti-platelet antibodies and thus immune. This project was performed to see if platelet increments could be improved by providing crossmatch compatible platelets for haematology patients with non-immune platelet refractoriness in addition, to provide more information on the possible effect of complement on platelet activation and destruction. P-selectin and complement markers C1q, C3 and C3dg were compared in patient's pre and post transfusion and in apheresis and whole blood derived platelet donations to identify any association with poor platelet increments. The results showed that the provision of crossmatch compatible platelets did not improve platelet increments. Comparison of the two platelet manufacture methods showed that apheresis platelets had higher levels of P-selectin and complement components C1q and C3 on them. However, this did not account for the poor increments or incompatible crossmatches.

# Acknowledgements

I would like to thank my supervisors Dr Nina Dempsey-Hibbert and Dr Wayne Thomas for all their help and support. Dr Thomas Scorer recently joined us as a member of staff, however, he has been instrumental in helping with the general layout and tidying up of this work and I am extremely grateful for his input. Due to the pandemic, I was unable to go to the ward myself to explain this work to the patients and consent them If they were willing so I would like to thank Caroline Lowe, our specialist transfusion practitioner for her help and input here. The research and development department at University Hospitals Plymouth were a great help where it came to gaining ethics approval and completing documents – especially Hannah Allende, Helen Neilens and Victoria Yates. I would also like to thank the blood bank manager Martin Binney for all his support during the HSST course and all my work colleagues who have stood in for me when I have been completing this work.

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S. J. Reynolds

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# List of Abbreviations

| | |
|---|---|
| ADP | Adenosine diphosphate |
| ATD | Adult therapeutic dose |
| ATP | Adenosine triphosphate |
| BD | Becton Dickinson |
| CCI | Corrected count increment |
| CMP | Common myeloid progenitors |
| c-mpl | Cellular homologue of the myeloproliferative leukaemia virus oncogene |
| CMV | Cytomegalovirus |
| CPB | Cardiopulmonary bypass |
| DIC | Disseminated intravascular coagulation |
| EDTA | Ethylenediaminetetraacetic acid |
| EGF | Epidermal growth factor |
| ELISA | Enzyme-linked immunosorbent assay |
| ENA-78 | Epithelial neutrophil activating peptide |
| FGF | Fibroblast growth factor |
| FN | False negative |
| FP | False positive |
| Gas6 | Growth arrest specific 6 |
| gC1qR | Globular C1q receptor |
| GMP | Granule membrane protein |
| GP | Glycoprotein |
| HCRW | Health and care research Wales |
| HEPES | (4- (2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| HGF | Hepatocyte growth factor |
| HLA | Human leucocyte antigen |
| HPA | Human platelet antigen |
| HRA | Health research authority |
| HSC | Haemopoietic stem cell |
| IGF | Insulin-like growth factor |
| IRAS | Integrated research application system |
| IgG | Immunoglobulin G |
| ITP | Immune thrombocytopenic purpura |
| LA | Latex agglutination |
| LCT | Lymphocytotoxicity tests |
| LISS | Low ionic strength saline |
| MASPAT | Monoclonal antibody solid phase platelet antibody test |
| MEP | Megakaryocyte progenitors |
| MFI | Mean fluorescence intensity |
| MMP | Matrix metalloproteinase |
| MP | Microparticle |

| | |
|---|---|
| MPP | Multipotent progenitors |
| MPV | Mean platelet volume |
| mRNA | Messenger ribonucleic acid |
| NHS | National health service |
| NHSBT | NHS Blood and Transplant |
| NPV | Negative predictive value |
| PADGEM | Platelet activation dependent granule-external membrane |
| PAI-1 | Plasminogen activator inhibitor |
| PDGF | Platelet derived growth factor |
| PF4 | Platelet factor 4 |
| PIFT | Platelet immunofluorescence test |
| PNH | Paroxysmal nocturnal haemoglobinuria |
| PPV | Positive predictive value |
| PRAT | Platelet radiolabelled antiglobulin tests |
| PRP | Platelet rich plasma |
| PSGL-1 | P-selectin glycoprotein ligand |
| PWSS | Platelet wash and storage solution |
| RANTES | Regulated on activated normal T expressed and secreted |
| SD | Standard deviation |
| SDF-1α | Stromal cell derived factor 1 |
| SLE | Systemic lupus erythematosus |
| TGF-β | Transforming growth factor β |
| TN | True negative |
| TP | True positive |
| TPV | Total predictive value |
| TRALI | Transfusion related acute lung injury |
| TXA2 | Thromboxane A2 |
| UHP | University Hospital Plymouth |
| UK | United Kingdom |
| USA | United States of America |
| VEGF | Vascular endothelial growth factor |
| VOD | Veno occlusive disease |
| vWF | von Willebrand factor |

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# Chapter 1 Introduction

## 1.1 Platelets

### 1.1.1 Platelet Structure

Platelets are the smallest cell type in the blood (2-3µm) and appear normally as biconvex discs. They lack a nucleus but do have a lifespan of approximately 10 days because of protein synthesis due to residual messenger ribonucleic acid (mRNA) in megakaryocytes.

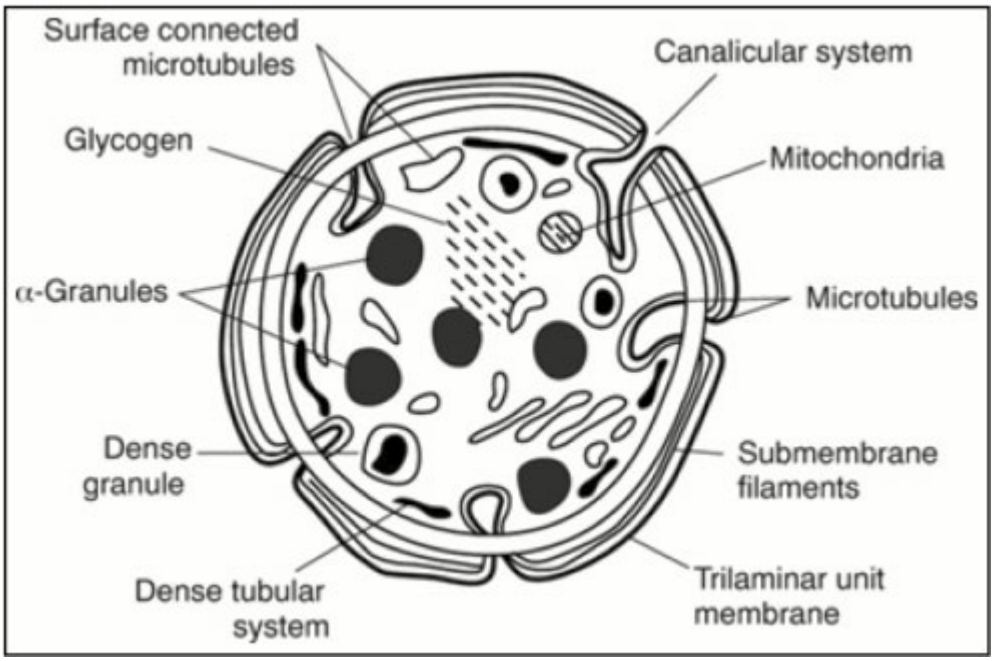


*Figure 1.1: Platelet structure from Mohan et al., 2019 showing the various organelles present in platelets and the membrane structure.*

Figure 1.1 shows the basic structure of a platelet which includes a network of interconnected channels, the open canalicular system, that extends from the inside of the platelet to the outside environment and may function to allow the rapid release of the constituents of platelet granules. Mitochondria produce ATP (adenosine triphosphate) and may also participate in the regulation of the platelet activation response. The platelet

plasma membrane, composed of a phospholipid bilayer, is the site of expression of various surface receptors and lipid rafts which helps in signalling and intracellular trafficking. The highly specialised cytoskeleton maintains its discoid structure as well as protecting the cell from being sheared in the blood stream. It has three major components: the spectrin-based membrane skeleton, the actin cytoskeleton, and the marginal microtubule coil (Ghoshal and Battacharyya 2014). Platelets contain four types of storage granule, dense granules, α-granules, lysosomes and peroxisomes, and several mitochondria. The dense granules contain high levels of ADP (adenosine diphosphate), ATP, phosphates, serotonin (for vasoconstriction), and calcium ions. The α-granules contain a rich diversity of proteins and membrane receptors that support haemostasis, vascular repair, inflammation (Wagner 2005) and host defence and these are listed in Table 1.1.

*Table 1.1: Platelet α-granule constituents and their physiological role (Taken from Hoffebrand et al. 2015 p.700).*

| **Physiological role** | **Constituent** |
|---|---|
| Angiogenesis | VEGF-A, VEGF-C, PDGF |
| Antibodies | IgG |
| Coagulation cascade | Factor V, fibrinogen, tissue factor pathway inhibitor |
| Endothelial cell activation | TGF-β |
| Fibrinolysis | Plasminogen, PAI-1, $\alpha_2$-antiplasmin |
| Growth factors | PDGF, FGF, HGF, IGF-1, EGF |
| Leucocyte recruitment | Chemokines, PF4, RANTES, β-thromboglobulin, ENA-78, SDF-1α |
| Matrix breakdown | Hydrolytic enzymes MMP-2, MMP-9 |
| Membrane proteins | GPIIb/IIIa (integrin αIIbβ3), P-selectin, CD40L |
| Bacterial killing | Microbicidal proteins |
| Miscellaneous | Amyloid β-protein precursor, Gas6 |
| Proteases | Protease nexin II |
| Platelet aggregation | Fibrinogen, fibronectin, vWF |
| Irreversible aggregation | Thrombospondin (locks fibrinogen bridges between GPIIb/IIIa (integrin αIIbβ3)) |

## 1.1.2 Platelet production by the bone marrow

Haematopoiesis is the lifelong process of continuous formation and turnover of blood cells to meet everyday demands as well as respond to increased need, for example, injury or

infection. The average human adult produces approximately one trillion blood cells every day (Doulatov et al., 2012). The predominant site of haematopoiesis is the bone marrow where multipotent haemopoietic stem cells (HSC) differentiate into myeloid or lymphoid cell lines. Thrombopoiesis (platelet production) occurs in the bone marrow and multipotent progenitors (MPP) differentiate into common myeloid progenitors (CMP) and the megakaryocyte progenitors (MEP). These MEPs then differentiate into megakaryocytes from which platelets are ultimately formed. Thrombopoiesis is mainly regulated by the hormone thrombopoietin (TPO) produced by the liver and kidney. TPO is the major physiologic regulator of megakaryocytopoiesis (production of megakaryocytes) and platelet production and binds to its specific receptor, c-mpl (cellular homologue of the myeloproliferative leukaemia virus oncogene). This leads to receptor dimerization, activation of intracellular signal transduction pathways, and responses of target cells (Ng et al., 2014). TPO and its receptor, c-mpl, are the primary regulators of platelet production and critical for hematopoietic stem cell (HSC) maintenance (Hitchcock et al., 2021). Upon binding to the c-mpl receptors on platelets and megakaryocytes, TPO is removed from the circulation and destroyed, which reduces blood levels. In summary, an autoregulatory loop is established: as platelet counts rise, they remove more TPO from the circulation, whereas in thrombocytopenic states there are less platelets to adsorb TPO, allowing levels to rise and increase thrombopoiesis (Kuter et al., 2009). It is now becoming evident that megakaryocytopoiesis does not occur as a stepwise process but is dynamic and adaptive to biological need and these new studies are suggesting that hematopoietic stem cells originate as megakaryocyte-primed and bypass the traditional hierarchical process (Noetzli et al., 2019). Thrombocytopenia is where there is a lower platelet count than normal (<150 X $10^9$/L) which is due to either a decreased production or increased destruction or

consumption. Although the exact pathogenesis of prolonged thrombocytopenia remains unknown, there are two primary theories as to its origin: destruction in the peripheral circulation and deficiency of production in the bone marrow (Zhang et al., 2015).

## 1.2 Haemostasis

Haemostasis is a complex system (Figure 1.2) of activating and inhibitory pathways that integrate five major components – blood vessels, platelets, coagulation factors and inhibitors and fibrinolytic elements (Hoffbrand et al. 2015, p.676). This complex system takes place to minimize blood loss and repair vessel injury and includes platelet activation and plug formation; assembly and activation of the coagulation cascade factors; fibrin clot formation at the site of injury; and dissolution of the clot and vascular repair (Cardenas 2016).

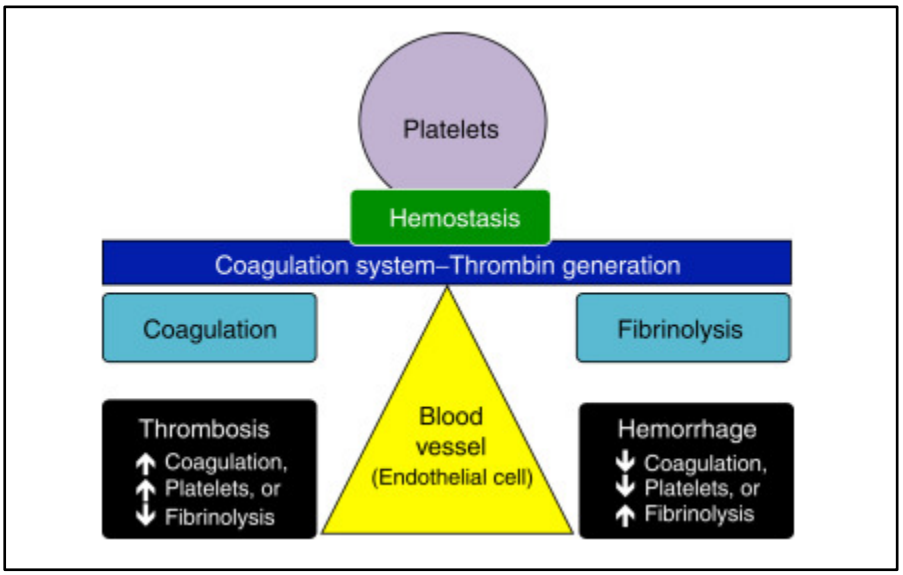


*Figure 1.2: Balance of haemostasis: the interaction of blood platelets, vascular endothelial cells, and blood plasma-coagulation factors are needed for successfully achieving a blood clot (Taken from Overview of Blood Coagulation and the Pathophysiology of Blood P.716)*

The haemostatic pathway is tightly regulated and if the system becomes unbalanced it can result in either hyper or hypocoagulation (Figure 1.2). Other systems in the body, such as the innate immune system and the inflammatory response, are also closely related to blood clotting and are influenced by the activation of haemostasis.

### 1.2.1 Blood vessels

Blood vessels, especially their endothelial lining, play a critical role in the maintenance of vascular fluidity, arrest of haemorrhage, prevention of thrombosis and regulation of inflammatory cell processes. In healthy vessels, the endothelium expresses and secretes the vasoactive hormones nitric oxide and prostacyclin, both of which relax blood vessels and inhibit platelet activation (Sagripanti and Carpi 2000).

### 1.2.2 Coagulation

Damage to the blood vessel wall triggers coagulation, initiating interactions between platelets and blood coagulation factors. This leads to the formation of a fibrin-containing clot, which stops bleeding and initiates vessel repair. The coagulation cascade is divided into two main systems, the extrinsic and intrinsic pathways. Both these pathways, although separate, interact in the body, leading to the cleavage of prothrombin to thrombin which is in turn needed for the cleavage of fibrinogen to fibrin and the formation of a stable fibrin clot. These systems co-exist and are controlled by positive and negative feedback which constantly ensures equilibrium between procoagulant and anticoagulant properties. The exposure of subendothelial tissue factor to plasma factor VII, ultimately leads to cross-linked fibrin formation (Smith et al., 2015). Additional coagulation (clotting) factors beyond factor VII, such as Factor V, VIII, IX and XIII respond in a cascade to form fibrin strands, which strengthen the platelet plug formation (de Gaetano and Cerletti 2009). The cascade from

initiation by tissue factor follows down from the conversion of FVII to FVIIa which catalyses the conversion of FX to FXa and it is at this step that the extrinsic and intrinsic pathways meet to follow a common pathway, where FXa and FVa allow for the conversion of prothrombin to thrombin. The production of thrombin allows for the pivotal step of the formation of fibrin from fibrinogen and with the aid of FXIII to form a stable fibrin clot.

### 1.2.3 Fibrinolysis

Fibrinolysis is a process that prevents blood clots that occur naturally from growing and causing problems. It is initiated by the binding of fibrin to tissue plasminogen activator, which leads to the conversion of plasminogen to plasmin. Plasmin then proteolyzes insoluble fibrin to form fibrin degradation products (Longstaff and Kolev, 2015).

## 1.3 Platelets in haemostasis

The platelet is a multifunctional cell (Figure 1.3), but their main function is their role in haemostasis where they contribute to prevention of blood loss from a damaged vessel. Platelets have different glycoprotein (GP) receptors on them, and changes occur in these during and after platelet activation.

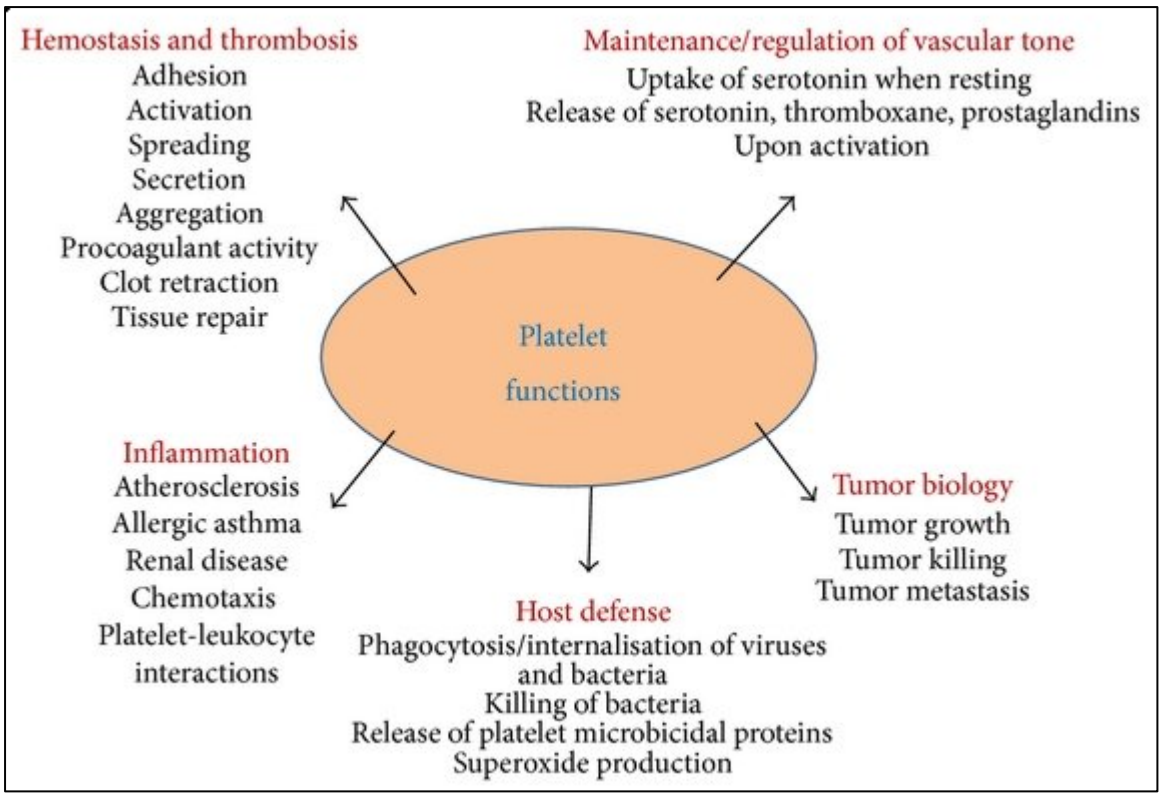


*Figure 1.3: Adapted from Harrison, (2005) showing the multifunctional platelet. This diagram shows that the platelet has many functions, and it is through these that certain medications can be used to enhance or inhibit them.*

### 1.3.1 Vascular phase

In the vascular phase, small blood vessels adjacent to the injury dilate (vasodilatation) and blood flow to the area increases. The endothelial cells initially swell, then contract to increase the space between them, thereby increasing the permeability of the vascular barrier – basically responsiveness of the blood vessel.

### 1.3.2 Platelet adhesion

The platelet adhesion mechanism is generally supported by the interactions between the membrane receptors and absorbed plasma proteins. The platelet membrane receptors are enriched with GP receptors embedded in the phospholipid bilayer (Figure 1.4), including

tyrosine kinase receptors, integrins, leucine rich receptors; G- protein coupled transmembrane receptors, selectins, and immunoglobulin domain receptors (Periayah et al., 2017).

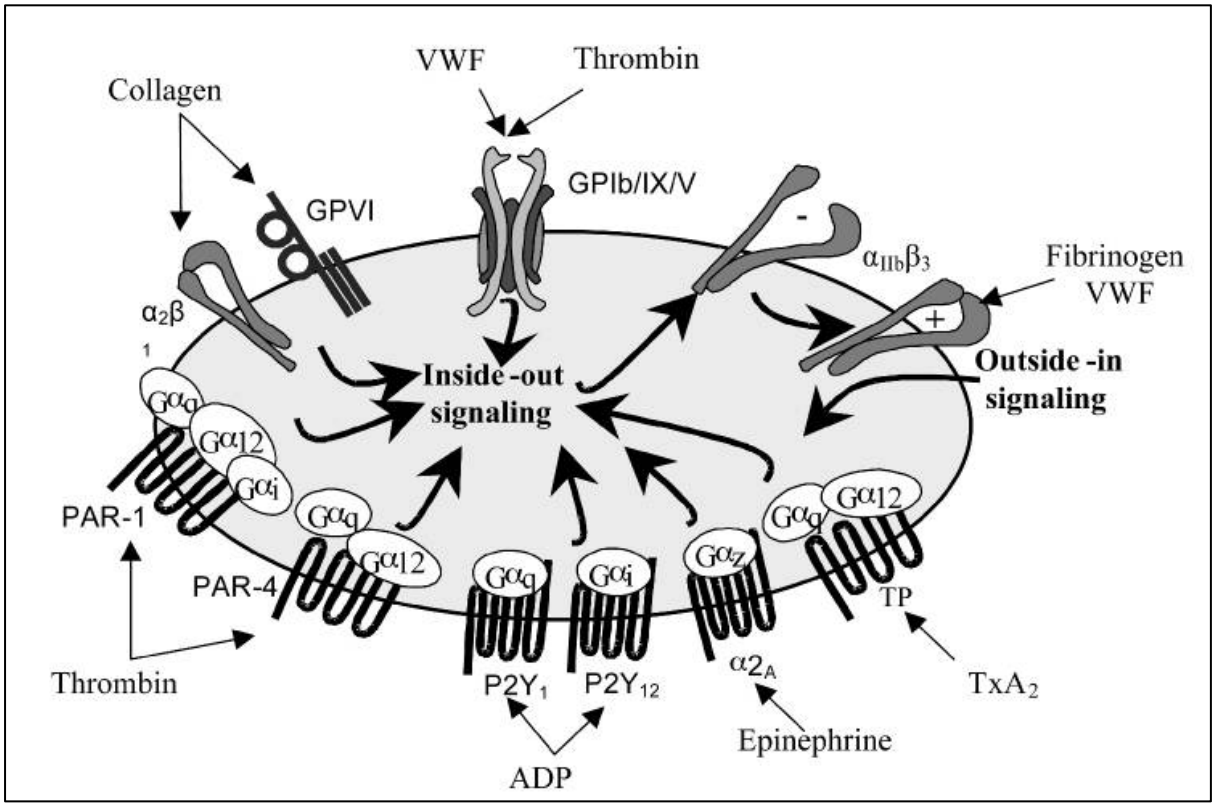


*Figure 1.4: Major platelet receptor-ligand interactions (Rivera et al., 2009) and their prominent role in the haemostatic function of platelets, allowing specific interactions and responses of vascular adhesive proteins and of soluble platelet agonists.*

Platelet thrombus formation *in vivo* is driven by cell–cell interactions facilitated by blood flow. Moreover, while forces exerted by rapid flow tend to detach adherent platelets, they can also promote adhesion by inducing conformational changes to shear-responsive adhesive proteins and platelet receptors, for example, vWF and GPIb, thereby facilitating and strengthening interactions (Panteleev et al., 2021). It should be noted that the shear rate can range from extremely low values (close to zero, or even reversed) to above

1100 $s^{-1}$ within large arteries and even higher in arterioles. Arteries experiencing high shear stress have an increased concentration of platelets, which accelerates the growth of the thrombus (Sakariassen et al., 2015) and adhesion depends on the long vWF which binds to exposed collagen and unfolds to expose its binding sites for GPIb-IX-V. During haemostasis, exposure of sub-endothelial collagen occurs after vessel injury which promotes binding of vWF, and this serves as a bridge between the collagen and the GPIb-V-IX complex on the platelet. The binding of the GPIb-IX-V complex to vWF mediates 'outside-in' signalling to initiate platelet spreading which is important in reducing drag caused by rapid blood flow and increasing the surface area that can interact with the sub endothelium (Ruggeri and Mendolicchio 2007). This consequently facilitates adhesion of platelets directly to collagen via platelet GPVI and GPIa/IIa (integrin α2β1) and the binding of GPVI also activates GPIIb/IIIa (integrin αIIbβ3) which binds to vWF and fibrinogen (Nieswandt et al., 2001) which are important for firm platelet adhesion and aggregation (Figure 1.5).

Under low shear platelets can adhere directly to the sub endothelium without the need for vWF and GPIb-IX-V.

Figure 1.5 illustrates the chain of events in platelet function starting with adhesion to the sub endothelium and ending with platelet plug formation.

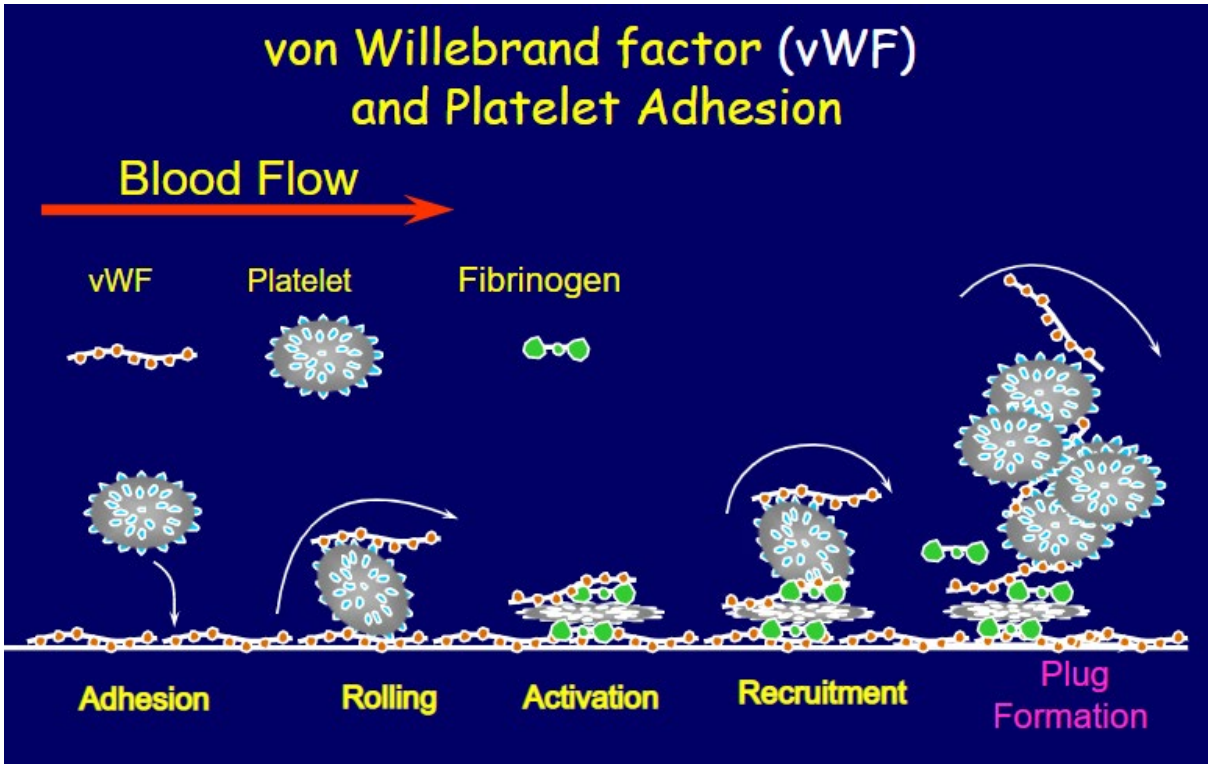


*Figure 1.5: Taken from a presentation on platelet structure and function from Dr.Mohammed Alotaibi showing the platelet functions from adhesion to the vessel via vWF, activation and aggregation and final platelet plug formation.*

### 1.3.3 Platelet activation

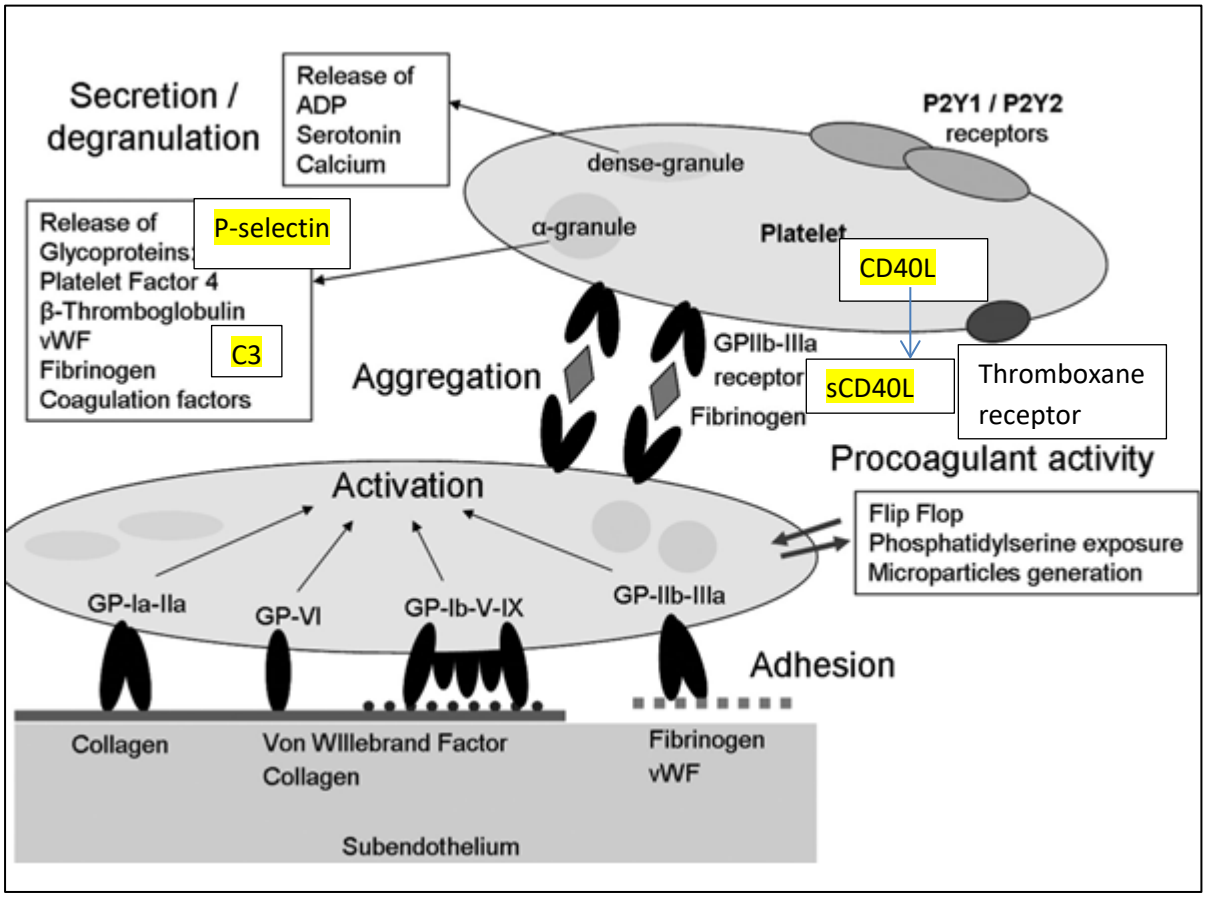


*Figure 1.6: The role of platelets in primary haemostasis (Adapted from Schoorl et al 2013). This diagram shows the platelet glycoproteins and their interactions during adhesion, activation, and aggregation. P-selectin, C3 and CD40L have been added to this representation as they will be discussed later in the introduction.*

Platelets circulate in the bloodstream in an inactive state but can undergo rapid activation as and when necessary. The adhesion of platelets to the sub endothelium leads to platelet intracellular signalling which activates the cell. Adhered platelets undergo degranulation and release α-granules that contain vWF and coagulation factors, such as fibrinogen, factor V, factor XIII and P-selectin (Table 1.1). Dense granules release platelet agonists, such as serotonin and ADP which play an essential part in normal haemostasis and thrombosis (Periayah et al., 2017). Figure 1.6 illustrates the different functional stages that platelets

undergo, and the various GPs involved in this process. P-selectin, C3 and CD40L have been added for completeness as these will be discussed later. Platelets are activated to change shape into a pseudopodal form upon the adhesion to the injured area which will activate the collagen receptor, GPIIb/IIIa (integrin αIIbβ3) on their surface membrane to undergo release reactions (Figure 1.7).

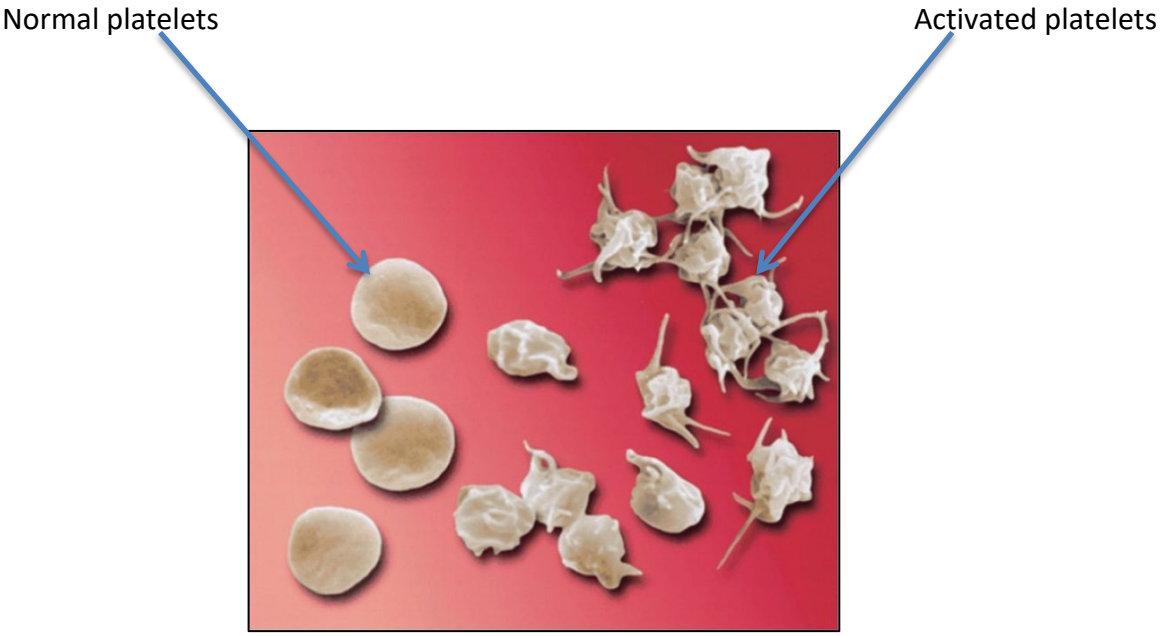


*Figure 1.7: Image of resting, partially activated and fully activated platelets (Taken from Kroll and Afshar-Kharghan 2012) to illustrate the shape changes that occur during the transformation from inactive to activated platelets.*

Activated platelets secrete thromboxane A2 (TXA2), which is a prostaglandin formed from arachidonic acid and serotonin and these are vasoconstrictors decreasing blood flow to the injured vessel. TXA2, serotonin and ADP released also activate other platelets. The resultant shape change that occurs due to platelet activation and exposure of P-selectin (CD62P) on the platelet membrane results in a procoagulant platelet surface (Schoorl et al., 2013). Activated platelets provide a negatively charged phospholipid surface for the assembly of

the tenase and prothrombinase complexes mediated by a rise in intracellular calcium ions. This is important for amplification of the clotting cascade, enhancing platelet activation and conversion of fibrinogen to fibrin.

Expression on the external platelet surface of GPIIb/IIIa (integrin αIIbβ3), GPVI, and P-selectin increases with activation, whereas that of GPIb-IX decreases.

The MPV (mean platelet volume) changes upon activation and this parameter has been suggested as a potential measure of platelet activation (Gasparyan et al., 2010; Korniluk at al., 2019).

### 1.3.4 Platelet aggregation

Platelets adhere to each other via adhesion receptors (integrins) forming a haemostatic plug with fibrin. Following platelet adhesion and activation GPIIb/IIIa (integrin αIIbβ3) undergoes a conformational change through 'inside out' signalling and binds fibrinogen. This interaction results in the formation of platelet aggregates, ultimately forming a platelet plug that can prevent bleeding (Periayah et al., 2017). This is maintained by continued signalling of fresh platelets arriving at the thrombus site. Myosin and actin filaments in platelets are stimulated to contract during aggregation further reinforcing the plug and help release of granule contents and this is clot retraction which stabilises the clot.

## 1.4 Mechanisms of platelet clearance – normally and during pathology

Known platelet clearance mechanisms include antibody-mediated clearance by spleen macrophages, platelet consumption due to massive blood loss, and apoptosis-induced platelet clearance (Grozovsky et al., 2010). Deglycosylated platelets are recognized by the

Ashwell-Morell receptor and potentially other scavenger receptors and are rapidly cleared by hepatocytes and/or macrophages. Overall, common mechanisms of platelet clearance have begun to emerge, suggesting potential strategies to extend the shelf-life of platelets stored at room temperature or to enable refrigerated storage (Quach et al., 2018).

Haemostatic disorders in the context of graft versus host disease (GVHD) are significant complications with multifactorial etiology, including tissue injury with releasing microparticles, cytokine release, macrophage/monocyte clearance, cytomegalovirus (CMV) infection, production of transforming growth factor-beta, and low levels of thrombopoietin. Future clinical trials with agents that stimulate megakaryocytopoiesis or influence underlying impaired haemostasis mechanisms should investigate whether such interventions may improve outcomes in patients with GVHD (Pulanic et al., 2009).

## 1.5 Platelets – essential components of the immune system

Platelets contribute to diverse immunological processes including intervention against microbes; recruitment and promotion of innate effector cell functions; modulating antigen presentation and enhancing the adaptive immune response (Ali et al., 2015). Platelets contain several complement factors and can interact with *Escherichia coli* and upon contact with bacteria, platelets are activated and can enhance complement activation (Arbesu at el., 2016).

## 1.6 Complement and platelet destruction

It is likely that most of the C3 is stored in α-granules (Figure 1.6) and that an increase in C3 upon platelet activation represents deposition of the protein on the platelet surface (Del Conde et al., 2005).

Various proteins of the complement system interact with platelets, which can lead to their activation. Activated platelets become opsonised by circulating complement, namely C1q and C3b which enables macrophages to recognise them and remove them by phagocytosis (Labrie 2018).

Complement is part of the innate immune system and consists of a series of serine proteases which activate each other in a sequence known as a cascade. There are three different complement pathways: the classical complement pathway, the alternative complement pathway, and the mannose-binding lectin pathway. Only the former two pathways will be considered here. The classical complement pathway is triggered when antibody-antigen complexes interact with the C1-complex, which consists of C1q, two molecules of C1r, and two molecules of C1s (Figure 1.8).

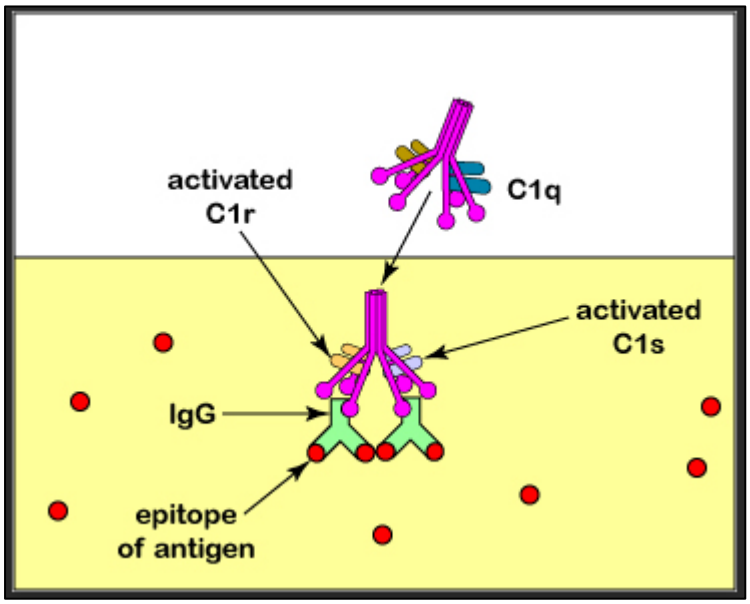


*Figure 1.8: Taken from Kaiser 2014 - lecture on activation of C1 during the classical complement pathway. The Fab (fragment antigen binding) of 2 molecules of IgG or 1 molecule of IgM bind to epitopes on an antigen. C1, consisting of C1q, C1r, and C1s then binds to the Fc (fragment crystallising) region of the antibodies. The binding of C1q to the antibody molecules activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b and complement protein C2 into C2a and C2b.*

The alternative complement pathway is directly initiated by C3 and does not require antigen-antibody interactions. The classical and alternative pathways are initiated differently but have the same endpoint (Figure 1.9).

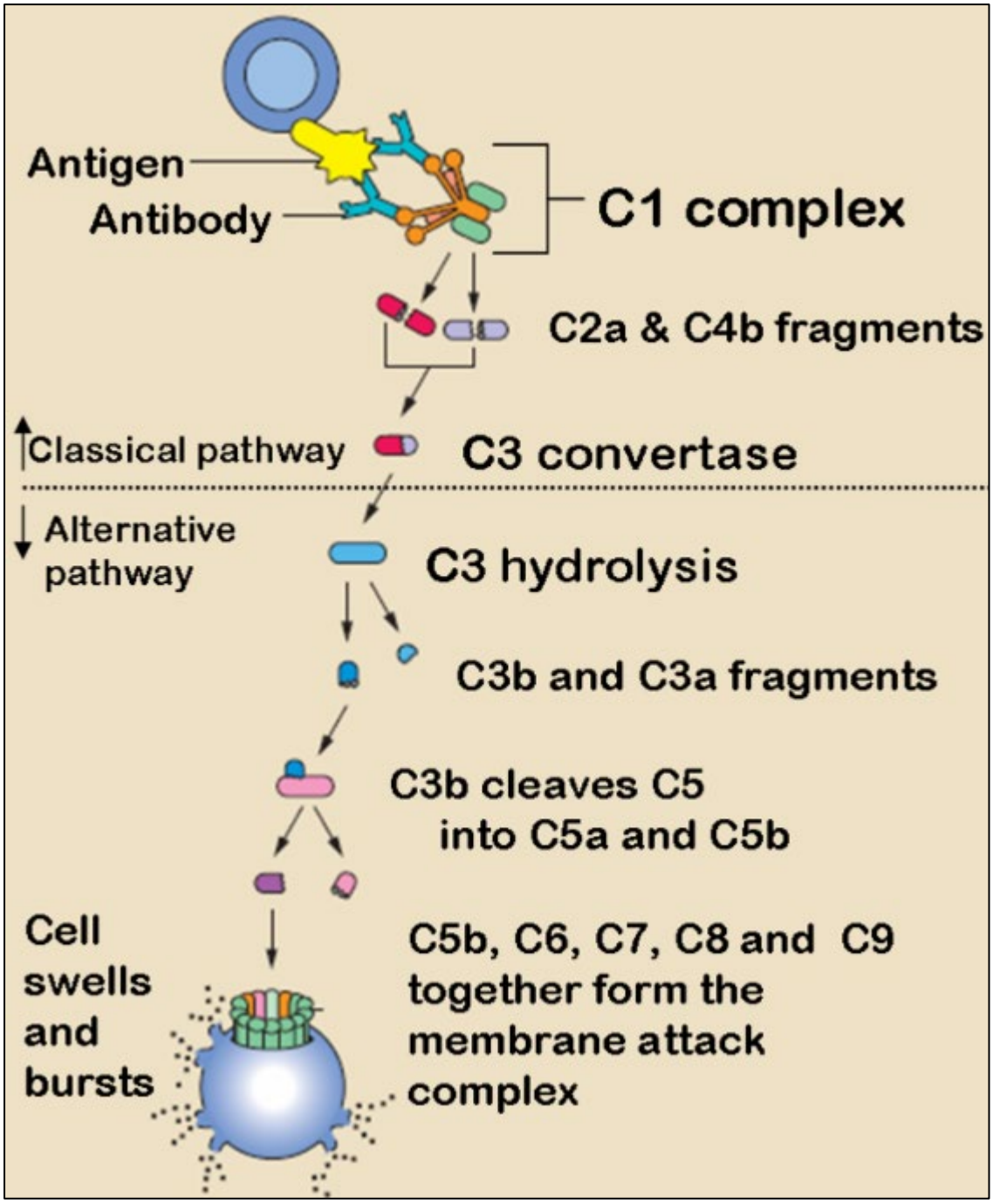


*Figure 1.9: Taken from Libretexts (2020) The Classical and Alternative Complement Pathways: The classical and alternative complement pathways start off differently, but end in the same cascade of complement proteins that combine to form a membrane attack complex.*

Complement appears to be involved in the destruction of platelets in certain clinical disorders, such as quinidine purpura and post-transfusion purpura (Shattil et al., 1978).

## 1.7 P-Selectin biology

P-selectin is present in the α-granules of platelets and the weibel-palade bodies of endothelial cells and is a member of the selectin family of cellular adhesion molecules. It is also known as GMP-140 (granule membrane protein of 140kDa based on molecular mass) and PADGEM (platelet activation dependent granule-external membrane) protein. During platelet activation the increase in the expression of P-selectin is accompanied by a simultaneous increase in the expression level of plasma soluble P-selectin, which plays an important role in the initiation, formation, and expansion of the thrombus. This shedding from platelets is thought to be a main source of the soluble form found in plasma following thrombotic events (Dunlop et al., 1992; Michelson et al., 1996).

### 1.7.1 Leucocyte recruitment

Fusion of α-granules leads to expression of P-selectin on the platelet surface, which is the major ligand for P-selectin glycoprotein ligand (PSGL)-1 on circulating neutrophils, monocytes, and lymphocyte (McEver & Cummings 1997) (Figure 1.10).

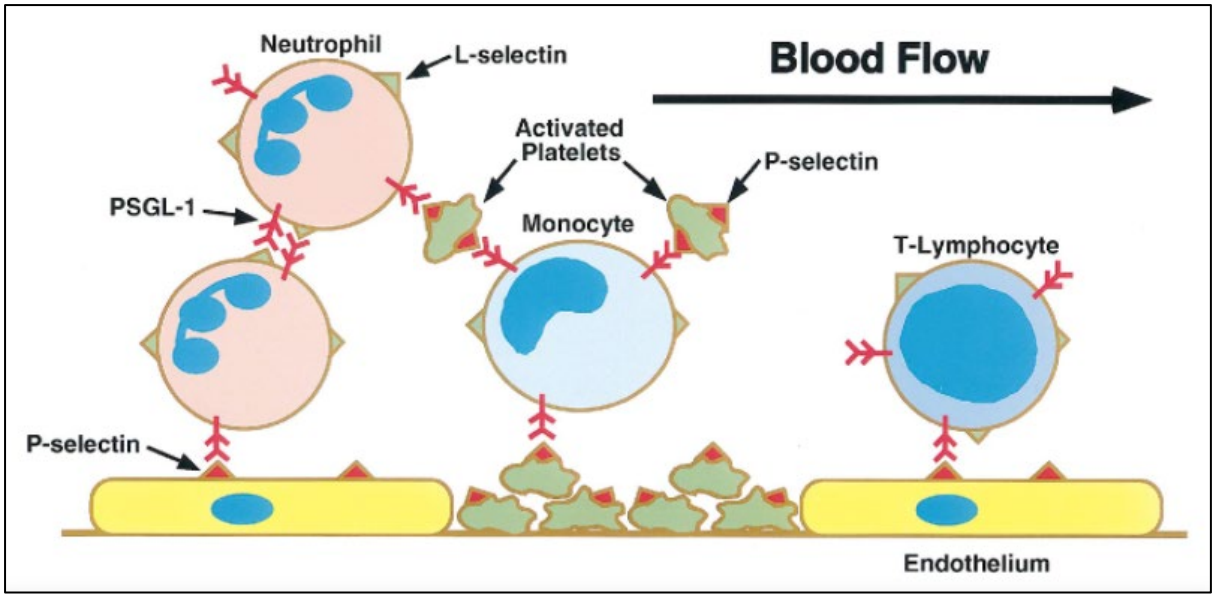


*Figure 1.10: Binding of PSGL-1 to P-selectin promotes tethering and rolling of leucocytes on activated endothelial cells and platelets. Binding of PSGL-1 to L-selectin (cell adhesion molecule found on leucocytes) mediates tethering of leucocytes to other leucocytes, which may amplify recruitment to the vascular wall (Taken from McEver and Cummings 1997)*

PSGL-1 plays a critical role in the tethering of these white cells to activated platelets or endothelia expressing P-selectin.

CD40L is localised in the platelet cytoplasm (Figure 1.6) and is a multifunctional protein belonging to the tumour necrosis factor superfamily and is expressed in a variety of cells (Skripchenko et al., 2008). Both soluble CD40L and soluble P-selectin are inflammatory markers and considered to be indicative of platelet metabolism and activation.

It has been well recognised that platelets are responsible for promoting procoagulant activity, but it has only been more recently that links have been found with inflammation. For example, (Lood et al., 2012) found that increased complement deposition on platelets maybe a valuable marker for platelet activation and venous thrombosis in SLE (systemic lupus erythematosus). Much earlier, (Myers et al., 1982) found a subgroup of patients with

immune thrombocytopenic purpura (ITP) that had elevated levels of platelet associated C3 which reduced platelet survival.

## 1.8 Platelet disorders

Platelet dysfunction may be due to a problem in the platelets themselves or to an external factor that alters the function of normal platelets.

### 1.8.1 Congenital platelet disorders

Inherited platelet disorders comprise a heterogeneous group of rare disease caused by molecular anomalies in genes that are relevant in platelet formation and/or function (Palma-Barqueros et al., 2021).

#### 1.8.1.1 Disorders of adhesion

Bernard Soulier syndrome is an autosomal recessive disorder characterised by moderate or severe thrombocytopenia and giant, dysfunctional platelets due to defects in the GPIb-IX-V complex which is required for adhesion of the platelet to the sub endothelium.

#### 1.8.1.2 Disorders of aggregation

Glansmanns Thrombasthenia is an autosomal recessive disorder due to a reduced or deficient GPIIb/IIIa (integrin αIIbβ3) which enables platelet aggregation through fibrinogen bridges. Heterozygote individuals tend to be asymptomatic with homozygotes exhibiting moderate severe mucocutaneous bleeding.

#### 1.8.1.3 Disorders of granules

Platelet storage pool deficiencies occur when platelet granules are absent, reduced in number, or unable to empty their contents into the bloodstream. Examples of these are

gray platelet syndrome, storage pool deficiency, Hermansky-Pudlak syndrome and Chediak-Higashi syndrome.

### 1.8.2 Acquired platelet disorders

Cardiopulmonary bypass (CPB) activates platelets with resultant structural and biochemical changes. Alterations in platelet count, clot formation, and the way in which platelets interact with other cells is also seen. Other acquired platelet disorders are seen in patients with disseminated intravascular coagulation (DIC) who have low or rapidly decreasing platelets and in uraemic patients with renal failure who have platelet dysfunction partially due to uraemic toxins present in circulating blood.

Antiplatelet drugs used to prevent thrombus formation in strokes and heart attacks and their reoccurrence by reducing the stickiness of platelets are included in the acquired platelet disorder group. They can also help alleviate symptoms of chest pain, poor circulation, and shortness of breath. The antiplatelet agents currently under development include thrombin receptor antagonists, phosphodiesterase inhibitors, a thromboxane–prostaglandin receptor antagonist, a serotonin receptor blocker, a platelet adhesion antagonist, nitric oxide-releasing aspirin, a glycoprotein VI antagonist, and a cyclooxygenase inhibitor. Table 1.2 shows a list of the antiplatelet drugs used and their mode of action.

*Table 1.2: Antiplatelet drugs and their mode of action (Das et al., 2010)*

| Class | Drugs |
|---|---|
| Cyclooxygenase inhibitors | Aspirin |
| | Indobufen |
| ADP receptor antagonists | Ticlopidine |
| (Thienopyridines) | Clopidogrel |
| | Prasugrel |
| ADP receptor antagonists | Cangrelor |
| (Nonthienopyridines) | Ticagrelor |
| | Elinogrel |
| Glycoprotein IIb/IIIa inhibitors | Abciximab |
| | Tirofiban |
| | Eptifibatide |
| | Defibrotide |
| Phosphodiesterase inhibitors | Dipyridamole |
| | Cilostazol |
| | NT-702 (parogrelil hydrochloride, NM-702) |
| Protease-activated receptor (PAR-1) | SCH 530348 |
| inhibitors (thrombin receptor inhibitor) | E5555 |
| Thromboxane $A_2$ receptor inhibitor | Terutroban (S18886) |
| Platelet adhesion antagonist | ARC1779 |
| | C1qTNF-related protein-1 |
| | DZ-697b |
| Nitric oxide releasing aspirin | NCX-4016 |
| Collagen-platelet interaction inhibitor | PR-15 |
| | Monoclonal antibodies |
| | Aptamers |
| | Small molecule peptide inhibitors |

## 1.9 Platelet refractoriness

Although platelet transfusions are vital to the ongoing management of many patient groups, it has been reported that up to 35% of haematological malignancy patients become refractory to platelets during their treatment (Millar, 2018). Due to the significant number of patients becoming refractory to platelet transfusion prompt investigation of the cause will encourage appropriate selection of platelet components. Phekoo et al., (1997) found that increased testing for leucocyte and platelet antibodies resulted in reduced demand for and more selective use of HLA-compatible platelets, with no apparent increase in haemorrhagic complications and that an audit of platelet refractoriness by found that clinicians differed in their definition of platelet refractoriness, and non-immune factors were not considered as important as immune causes.

Platelet refractoriness is defined as a poor response (immediate or 24-hour increment post platelet transfusion of <10 $x10^9$/L) to random donor platelets on two or more occasions. Platelet refractoriness itself is known to be associated with several adverse outcomes including prolonging hospital stays along with higher in-patient hospital costs (Meehan et al, 2000), increased risk of bleeding and decreased survival (Kerkhoffs et al, 2008).

Most of the literature focusing on the success of platelet transfusions in raising the platelet count post-transfusion (platelet increments) used the corrected count increment (CCI) calculation which was calculated as follows:

Platelet increment x $10^9$/L × body surface area ($m^2$) /number of platelets transfused (× $10^{11}$) at 20 hours.

One unit of platelets transfused is taken as 3 x $10^{11}$/L which is the mean value for each donation. A successful post transfusion count is taken to be >10 X $10^{9}$/L or a CCI of 5000 (Slichter et al 2005).

The typical platelet responses show the intended response in green (Figure 1.11), which illustrates that whilst a proportion of the platelets are removed from the circulation within the first 24 hours, the rest survive. However, there are still a few that have no obvious reasons for the continued refractoriness despite numerous transfusions.

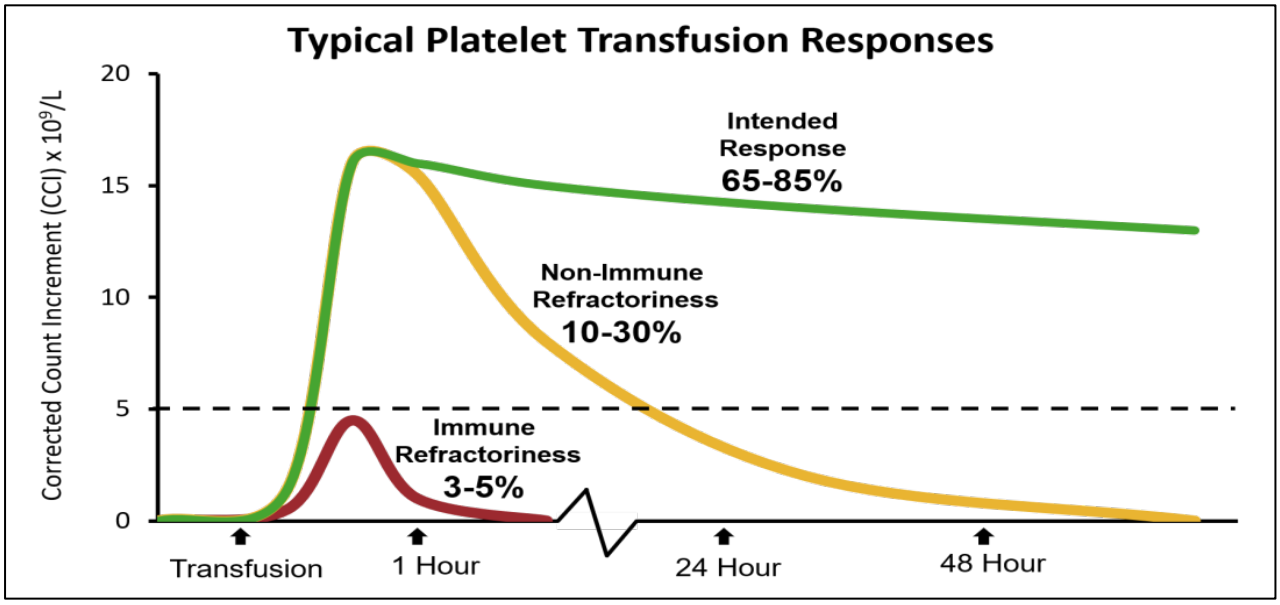


*Figure 1.11: Typical platelet transfusion responses (taken from Millar, 2018). The red line on immune refractoriness is the production of detectable antibodies known as alloimmunisation which accounts for 3-5% of patients (Slichter, 1998). The yellow line on the graph represents platelet transfusions that have produced inadequate platelet counts (10-30%) in the absence of immune refractoriness suggesting that other mechanisms are responsible for the platelet destruction.*

## 1.9.1 Etiology

Platelet refractoriness can occur due to either immune (antibody-mediated) or non-immune mechanisms. Platelet refractoriness is the main clinical problem associated with repeated platelet transfusions. This is most frequently due to HLA alloimmunisation, or non-immune

platelet consumption associated with clinical factors such as septicaemia (Murphy et al., 1994).

Alcorta et al., (1996) found that HLA alloimmunisation accounted for less than 33% of refractory patients and fever and bone marrow transplant because of chronic myeloid leukaemia were the only non-immune factors independently associated with refractoriness. Despite being a frequent condition, platelet refractoriness is still managed with a high degree of heterogeneity and often overlooked. An email survey was carried out in Italy, and it was concluded that better adherence to existing guidelines and standard operating procedures, as well as the involvement of transfusion centres in prospective evaluations can help reduce this variability and improve the outcome of transfused patients (Quaglietta et al., 2012).

### 1.9.2 Non-immune causes

Non-immune refractoriness is thought to account for 60-80% of cases (Forest & Hod 2016) and these include clinical conditions and possibly platelet characteristics. Non-immune causes, based on studies in patients with acute myeloid leukaemia (AML) or haemopoietic stem cell transplants, include fever, sepsis, splenomegaly, disseminated intravascular coagulation (DIC), bleeding, veno-occlusive disease (VOD), GVHD and medications (Ishida et al., 1998; Slichter et al., 2005). The non-immune factors associated with refractoriness are often multiple, most frequently a combination of fever, infection, and antibiotic therapy (Doughty et al., 1994). Transfusion service factors, such as storage of the platelet product and ABO incompatibility, also affect platelet increments (Hod and Schwartz 2008).

No single factor is a good predictor of response to platelet transfusion in each patient and

each patient may be sensitive to different factors (Friedberg et al., 1993).

#### 1.9.2.1 Sepsis

Platelets play key roles against infection and are involved in various mechanisms to promote the immune response and the activation of coagulation. Thrombocytopenia is common in the ICU (intensive care unit) during sepsis, causes are multiple, and low platelet count is correlated with poor outcome (Vardon-Bounes et al., 2019) caused by the dysregulated host response to infection. Sepsis management requires monitoring and intervention, often with an admission to the emergency department or intensive care if necessary. Thrombocytopenia is a risk marker and this significant decrease in platelet count should alert the clinician to correct the underlying cause.

#### 1.9.2.2 Fever

In haematology malignancy cases, the triad of fever, infections and medications is the most common cause of refractoriness (Doughty et al., 1994) and although fever produces reduced platelet increments, it is unclear whether this is due to infections and medications as well or fever alone. Petz et al., (2000) found that concurrent fever significantly reduced percentage platelet recovery when random donor platelets were transfused; however, fever did not significantly affect this when matched platelets were used.

#### 1.9.2.3 DIC

Disseminated intravascular coagulation (DIC) is an acquired syndrome characterised by activation of coagulation pathways, resulting in formation of intravascular thrombi and depletion of platelets and coagulation factors. Consumptive coagulopathy activates and immobilises platelets leading to poor CCI's following platelet transfusion (Bishop et al., 1988). Many of these will be due to the increased platelet consumption, however if the DIC is managed by treating the underlying cause this should be alleviated.

#### 1.9.2.4 Bleeding

Bleeding can be thought of as a non-immune cause of refractoriness but usually any bleeding is a consequence of the reduced platelet count itself which can be because of another cause of platelet refractoriness.

#### 1.9.2.5 Splenomegaly

As the spleen is the major site of platelet destruction, it is the major factor affecting platelet count increment (Slichter et al., 2005). It sequesters a large proportion of the platelet pool and in addition, hypersplenism is associated with a decreased time interval until the next transfusion.

#### 1.9.2.6 Haemopoietic stem cell transplant (HSCT), graft vs host disease (GvHD) and veno-occlusive disease (VOD)

Patients having allogeneic HSCT's are prone to alloimmunisation due to the amount of platelet support required in their treatment. There are also complications associated with this such as GVHD which may lead to increased production of anti-platelet autoantibodies and accelerated platelet destruction and the associated thrombotic microangiopathy (Anasetti et al., 1989). Prevalence of hepatic VOD in patients undergoing HSCT could account for platelet refractoriness.

#### 1.9.2.7 Drugs/medications

Drug-induced thrombocytopenia is usually immune-mediated with hundreds of drugs implicated in this process. An updated database of implicated drugs can be found at http://moon.ouhsc.edu/jgeorge/ditp.html (Terrell, D. 2015). Drug-induced thrombocytopenia does not require specific treatment other than the discontinuation of the sensitising agent. *In vitro*, amphotericin B induces P-selectin expression on the surface of inactivated platelets and increases platelet adhesion to polymorphonuclear neutrophils, which is exacerbated by

storage. Platelet dysfunction resulting from exposure to amphotericin B may contribute to poor platelet recovery in vivo when amphotericin B is administered concomitantly with platelet transfusion (Pastakia et al., 1996).

#### 1.9.2.8 Platelet characteristics

These non-immune mechanisms need to be ruled out first as a cause of low platelets before the immune mechanisms of platelet destruction are investigated (Brown 2011).

### 1.9.3 Immune causes

Many antigens present on platelets are also present on other blood cell types: for example, the ABH blood group antigens which are abundant on red cells are also found on platelets, while the HLA (human leucocyte antigen) class I which are cell-surface proteins responsible for the regulation of the immune system and expressed on all leukocytes, are inevitably expressed by platelets. HPA (human platelet antigens) are present on platelets and can cause platelet antibody formation.

#### 1.9.3.1 HLA (human leucocyte antigen)/HPA (human platelet antigen) alloimmunisation

Due to the polymorphic nature of the HLA and HPA, and the diversity across human populations, the production of HLA and HPA antibodies in an individual can be stimulated following the transfusion of HLA/HPA incompatible platelets. Among those diagnosed with immune refractoriness, there is no standard method for identifying platelet products likely to be effective (Vassallo et al., 2007), however, it seems that HLA- and HPA-alloimmunised patients can be successfully supported with HLA- and HPA-matched platelet concentrates (Kekomaki et al., 1998).

#### 1.9.3.2 ABO incompatibility

Valsami et al., (2015) reviewed the available data on transfusion practices and outcome in

ABO and RhD incompatibility and found that transfusion of platelets with major ABO-incompatibility was related to reduced post transfusion platelet increments compared to ABO identical and minor but were still equally effective in preventing clinical bleeding. An exclusive ABO-identical platelet transfusion seems to be the most effective and safest therapeutic strategy and could be feasible, but alternative approaches could facilitate inventory management.

Heal et al., (1989) studied the frequency of ABH incompatibility in platelet crossmatches and found that group O plasmas were incompatible with 52% of group A platelets and 17% of group B platelets. Transfusions of group B platelets to incompatible recipients may be more likely to yield satisfactory increments than incompatible transfusions of group A platelets, but this remains to be proven.

#### 1.9.3.3 Other causes

Many patients end up receiving daily platelet transfusions just to prevent bleeding due to low platelet counts but their platelet count never seems to increase. Investigation of platelet refractory patients in terms of HLA matching and platelet crossmatching has led to increased costs and difficulties in management with significant delays in treatment (Manis and Silberstein 2016). Treatment for non-immune platelet refractoriness, which is the most frequent cause, is often ineffective and is a complicated challenge (Solves Alcaina 2020). Each bag of platelets costs the NHS £220.58 and just a week of treatment could be as much as £1550 and that does not take into consideration consultant time, bed space and availability of donors (NHS blood and transplant portfolio of blood components 2021). Platelet donations have a shelf life of 5 days without bacterial monitoring and 7 days with once they have been processed from donor blood and should be stored at room temperature with constant agitation until transfusion to patients. If during this time they

become activated for some reason or they had already been previously activated in the donor circulation there is a possibility that they could be destroyed prematurely after transfusion. Platelets are known to become activated under different conditions and this activation can occur both in patients and platelet donations causing reduced platelet counts and compromising the quality of platelet donations. P-selectin exposure is known to be an established marker for platelet activation and this platelet activation status can be used for characterising quality and function of platelets in various experimental and clinical settings (Leytin et al 2000).

Various proteins of the complement system interact with platelets, which can lead to their activation. Activated platelets become opsonised by circulating complement, namely C1q and C3b which enables macrophages to recognise them and remove them by phagocytosis (Labrie 2018).

## 1.10 Manufacture of platelet concentrates

Standard platelet components are currently collected from donors either from whole blood donations or via an apheresis machine with an integrated leucoreduction system. The two different components are then produced via two manufacture methods by NHSBT clinical issue using the specifications in the red book - Guidelines for the Blood Transfusion Services in the United Kingdom, 8$^{th}$ Edition 2013 and are:

- A pool of buffy coat-derived platelets from four whole blood donations, suspended in 30 - 35% plasma and 65 - 70% platelet additive solution (PAS). The red book states that the volume of suspension medium must be sufficient to maintain the pH within the range 6.4-7.4 at the end of the shelf life of the component. This component is

labelled as platelets pooled in additive solution and plasma leucodepleted in citrate phosphate dextrose (CPD) anticoagulant.

- An adult therapeutic dose (ATD) obtained from a single donor by apheresis donation and anticoagulated with acid citrate dextrose (ACD), suspended in plasma.

Details of anticoagulants and PAS can be found in Appendix Table A3.

All platelets that have a 7-day shelf life have been bacteriologically screened in line with quality requirements and guidelines for bacterial screening and 5 days - if not bacteriologically screened. Platelets of all blood groups including AB are manufactured and stocked thus ABO and RhD identical units should be used as far as possible. Due to the population distribution of group AB and its value as a universal plasma donor, stocks may be limited at times. In terms of donor exposure there will be other potential disadvantages over apheresis platelets including increased donor exposure to infectious disease transmission risk and increased donor exposure to HLA (Chu et al., 2021).

### 1.10.1 Platelet storage lesions

New developments are required in the fields of platelet storage and platelet substitutes, and novel ways to avoid complications associated with platelet transfusions. Initiatives need designing to reduce inappropriate use of platelet transfusions so that we can preserve this precious resource for situations where there is evidence for their beneficial effect (Prodger et al., 2020).

Platelet storage lesions are a series of biochemical, structural, and functional changes that occur from blood collection to transfusion of platelets and understanding of these is crucial for devising interventions that prolong shelf life to improve access and wastage. Platelets get activated following exposure to foreign surfaces, such as those of platelet

apheresis machines, trauma, low pH, agonists (thrombin, ADP), and shear stress. ATP depletion occurs as ATP is required for each aspect of platelet function and is generated via oxidative and glycolytic pathways. Glycolysis is upregulated under low oxygen conditions and progressive lactate production and bicarbonate exhaustion during storage results in a deleterious decrease in pH augmenting platelet damage. Platelet apoptosis occurring simultaneously during storage further contributes to loss of mitochondrial function, cytoskeletal damage, and surface expression of phosphatidyl serine (Mittal and Kaur 2015). Platelet microparticles (MP) are cell-derived membrane vesicles that range in size between 0.1 and 1µm and originate from platelets and endothelial cells because of cell activation and apoptosis (Rank et al., 2011).

## 1.11 Platelet transfusion and clinical need

Due to the central role of the platelets in haemostasis, conditions in which there is a reduction in number (thrombocytopenia), or function (thrombocytopathy) of platelets can require the use of platelet transfusions and platelet transfusions are established as effective treatment for thrombocytopenic bleeding.

The demand for platelet donations increased due to a rise in the incidence and intensity of treatment for haematological malignancies and an aging population (Estcourt et al., 2016), however, this has plateaued in the UK in the last five years and fell during the COVID-19 pandemic. As haematology patients become refractory to platelet transfusions, they may have a daily need for platelet transfusions which will increase the chance of producing antibodies to HLA/HPA and thus exacerbating the refractoriness.

Guidelines have been produced for the use of platelet transfusions (Estcourt et al., 2016) and aim to provide practical advice on platelet transfusions to help clinicians decide when

support is expected to be beneficial and to reduce inappropriate use. If the reason for thrombocytopenia is unclear, further investigation is required as this is likely to influence management.

### 1.11.1 Prophylaxis and active bleeding

Platelet transfusions are used in modern clinical practice to prevent (prophylaxis) and treat bleeding in thrombocytopenic patients with bone marrow failure. The use of prophylactic platelet transfusions for the prevention of thrombocytopenic bleeding are an area of concern and a systematic review by (Crighton et al., 2015) found low to moderate grade evidence that a therapeutic only platelet transfusion policy was associated with increased risk of bleeding when compared with a prophylactic platelet transfusion policy. A therapeutic-only platelet transfusion policy is associated with a clear reduction in the number of platelet components administered which would prevent platelet refractoriness problems. Because of the intensive chemotherapy regimens that haematology patients go through as part of disease control or in preparation for a stem cell transplant, they almost always require extensive transfusion (blood component) support.

The effectiveness of platelet transfusions to prevent bleeding in patients with hematologic cancers remain unclear. A trial by (Stanworth et al., 2013) assessed whether a policy of not giving prophylactic platelet transfusions was as effective and safe as a policy of providing prophylaxis. The results of this study supported the need for the continued use of prophylaxis with platelet transfusion and showed the benefit of such prophylaxis for reducing bleeding, as compared with no prophylaxis. A significant number of patients had bleeding despite prophylaxis.

Dhiman et al., (2019) compared the efficacy of low and high dose single donor apheresis

platelets with standard dose transfusions and found they gave similar platelet responses and significantly reduced donor exposure.

## 1.12 Management of the refractory patient

Various methods are used to assess responses to platelet transfusions, the most common is measuring the increase in platelet count after transfusion, however, the clinical response is also an important indication of the effectiveness of the transfusion. All patients who receive multiple platelet transfusions should have their platelet count assessed on the day after transfusion on a regular basis to monitor their response and if platelet refractoriness occurs, platelet algorithms can be used for the investigation and management of patients (NHS Blood and Transplant clinical guidelines available at Microsoft Word - INF139.docx (windows.net). There are many different examples of these algorithms that have been adapted to define and recommend policies and procedures for the provision of optimal transfusion support for patient refractory to unselected platelet components and one such example can be seen below (Figure 1.12). These algorithms will be dependent on what services are available and can include HLA/HPA matched platelets and platelet crossmatching. Initial management of alloimmunised patients who are refractory to platelet transfusions from random donors is the use of HLA-matched platelet donors; this results in improved responses to platelet transfusions in about 65% of these patients (Murphy et al., 1990).

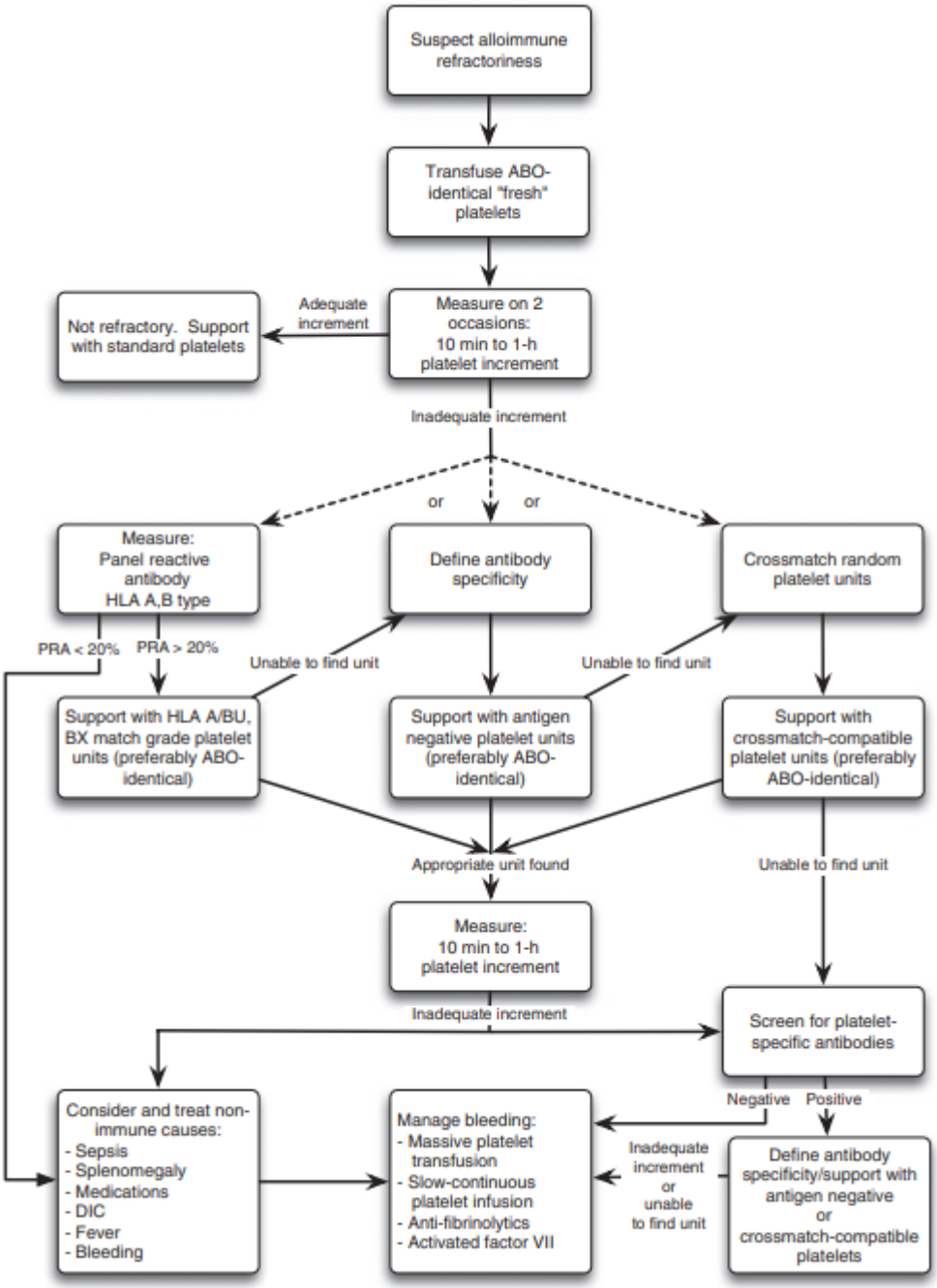


*Figure 1.12: An example of an algorithm taken from Hod and Schwartz (2008) that can be used for the investigation and management of platelet transfusion refractoriness. The panel reactive antibody (PRA) represents the percentage of individuals with whom the patient's HLA antibodies react; a PRA result of >20% suggests HLA alloimmunization.*

### 1.12.1 Options for platelet provision

Platelet crossmatching can be used to select compatible units for refractory patients, but un-like for red cell transfusions, platelet crossmatching is not performed routinely in many hospitals. The crossmatch process consists of mixing platelet donations with patient (recipient) plasma to see if any reaction occurs to mimic what may happen in-*vivo.* Platelet crossmatching is not routinely performed in the UK as the donations are issued to patients without any prior compatibility testing. In cases where HLA antibodies are detected HLA matched platelets are sourced from reference centres which can cause a delay to treatment whilst donors are located. However, if platelets were required urgently for a patient crossmatching could be used to ensure that compatible platelets were issued in a timely fashion rather than wait for the HLA matched platelets to arrive. Platelet crossmatching may reveal the presence of platelet-specific antibodies in some patients who are refractory to platelet transfusions from HLA-matched donors and may assist in the selection of compatible platelet donors. However, the identification of compatible donors is not possible in all refractory patients. Methodology for platelet crossmatching has been well studied and the different methods have been published widely in the literature. The impact of volume of platelet stock and its location is underplayed as it would not be practical for many hospital sites to hold large stocks of platelets for crossmatching due to their short expiry dates and storage method.

## 1.13 Overall objectives and aims of the thesis

As patients with a haematological malignancy become refractory to platelet transfusions, they can be tested for platelet antibodies at a reference centre and provided with HLA

and/or HPA-matched platelets if required. However, there are some patients who are still refractory in the absence of immune and non-immune factors.

The overall objective of this thesis was to evaluate platelet crossmatching for haematology patients to see if platelet increments correlated with a compatible platelet transfusion and if they did not to investigate whether P-selectin or complement expression on platelets could account for this.

This was achieved with the following objectives:

- Measurement of platelet counts in haematology patients to determine whether providing crossmatch compatible platelets increased the platelet count more readily than providing random donor platelets
- Measurement of P-selectin and complement markers C1q, C3, and C3dg on apheresis and pooled platelet donations to see if there was any correlation between these values and a compatible or incompatible platelet crossmatch
- Measurement of P-selectin and complement markers C1q, C3, and C3dg on haematology patients' platelets to see if the results correlated with their platelet refractoriness

The provision of compatible platelets for refractory haematology patients could reduce the morbidity and mortality rates, thereby enhancing quality of life and health, as well as reducing the number of donors required and the costs associated with the number of platelet donations. This work could contribute to further studies on the subject and influence any future guidelines on platelet selection for patients.

# Chapter 2 Literature Review

## 2.1 Introduction

This review chapter surveys the existing literature to evaluate current understanding in the methodologies and underlying biological processes that may affect successful platelet transfusion. This review will focus on three key areas:

I. Platelet crossmatching methodologies

II. Acquired causes of platelet activation

III. Complement and platelet biology

## 2.2 Methods

Searches were conducted using databases Embase and Ovid MEDLINE(R) 1978 to August 2022.

### 2.2.1 Search terms

The search terms used are in Table A34 of the Appendix.

### 2.2.2 Inclusion and exclusion criteria

Articles were included if they were:

- Human studies only
- Published in a peer reviewed journal
- Full text available via institutional accesses

Articles were excluded from the analysis if they were:

- Background articles or reviews

- Abstract only
- Non-English language full text article
- Published prior to 1978

### 2.2.3 Analysis of search results

Search results were exported to Endnote software, duplicates removed and screened against the inclusion and exclusion criteria. The full text of the shortlisted articles was then reviewed in more detail before arriving at the final selection.

## 2.3 Results

### 2.3.1 Summary of searches and article screening

A total of 236 articles were identified using the search terms described after duplicates had been removed and once articles failing to meet the criteria described in section 2.2.2 were removed this left 45 articles for full analysis.

### 2.3.2 Evaluation of platelet crossmatching

Many different methods of platelet crossmatching have been researched going back as far as the 1980's; however, there has never been a gold standard method introduced and crossmatching has not been mentioned in recommended guidelines due to the differing opinions on how useful it would be. Different practices exist for treating patient's refractory to platelet transfusions between and within countries. Maybe in the next few years this will change as platelet demand increases with the increasing number of transplants and the current practice of transfusing platelets every day in some cases just to raise the platelet count becomes unacceptable. There seem to be many papers on crossmatching platelets going back as far as the 1980's, however; hospitals in the UK are still not crossmatching

platelets routinely for patients that are refractory. Usually, samples are sent to reference centres to test for HLA and then HPA antibodies and if there are no antibodies detected then random donor platelets will continue to be issued to these patients with no further follow up. This practice should not continue as the demand for platelets cannot be maintained or justified. Even though platelet crossmatching has been researched since the 1980's it is still very rarely done in practice. The crossmatch methods for platelets studied in the research papers were varied and included platelet immunofluorescence tests (PIFT) using flow cytometry, Capture-P which is an ELISA (enzyme-linked immunosorbent assay) from Immucor, solid phase red cell adherence assay from Sanguin (MASPAT – Monoclonal Antibody Solid Phase Platelet Antibody Test), various in-house ELISA methods, platelet radiolabelled antiglobulin tests (PRAT), Lymphocytotoxicity tests (LCT) and latex agglutination (LA). PIFT involves incubating monoclonal antibody IgG conjugated to fluorescent probes with donor platelets and detecting with flow cytometry. Capture-P, ELISA and MASPAT are all the based around an ELISA test where a microtitre plate has platelet antigens bound to the wells – patient plasma is incubated with this, and any reaction is detected using sensitised red cells. PRAT is like PIFT, but a radiolabel is used instead for detection purposes. LCT with the addition of antiglobulin reagent improves the sensitivity of the assay for HLA antibodies and utilises lymphocytes as target cells which are incubated with pre-transfusion serum from the patient. Complement-dependent cytotoxic antibody present in the patients' serum is recognised by monitoring dye exclusion. LA is observed when a sample containing the specific antigen (or antibody) is mixed with an antibody (or antigen) which is coated on the surface of latex particles. The 1980's research tended to concentrate on PRAT, LCT, ELISA and PIFT. In the 1990's researchers were still using LCT, PIFT, ELISA, and LA – with the emphasis on PIFT and ELISA and moving into 2000 all research

involving antibody detection and crossmatch of platelets used solid phase techniques from various companies and PIFT only. A review of 18 papers was undertaken from 1978-2020 and the outcomes are listed in Table A1 of the appendix. From 1997-2020 all platelet crossmatching research was done using solid phase technology apart from in 2010 Sayed et al. (2010) used PIFT, however, 13 out of the 18 articles reviewed used solid phase technology with it first being used by Connell et al. in 1990. Various studies have looked at platelet crossmatching in comparison to HLA-matched platelets and found that most patients can be given crossmatch compatible platelets to improve outcome (Wiita and Nambiar 2012) however, there are still those that do not (Kickler, Ness et al. 1988). Jia, Li et al. (2014) also raised the issue regarding the number of platelet donations that maybe required before any compatible ones could be found and that this can become an issue in terms of staffing and time taken. O'Connell et al., (1995) used solid phase platelet crossmatching to support an alloimmunised patient with leukaemia and found that out of a total of 205 crossmatches only 11 were considered compatible. These 11 were then transfused during 5 transfusion episodes and 4 out of the 5 were considered successful. Supply of HLA-matched platelets can be difficult if the donors of a particular HLA type are unavailable. This practice in the UK is also limited to transplant centres where donors can be contacted, and their donation prepared for the product required. The disadvantages of this are the length of time it involves as donors are not always available. Many hospitals are large distances from processing centres, and this can cause delays to the transfusion of the patient. Chakrabarty and Das (2017) successfully used crossmatched platelets for a patient with aplastic anaemia where urgent surgical intervention was required.

Most of the crossmatch assays used platelet preparation methods as recommended by the manufacturer's instructions if using kits and these include MASPAT from Sanguin and Capture-P from Immucor. However, other in-house methods have devised their own platelet preparation methods. Only five of the literature sources mentioned the concentration of platelets used for their assays and these ranged from $10^8$ – $10^{11}$/L. Only (Vongchan, Nawarawong et al. 2008) fixed their platelets in paraformaldehyde, and this was a modified in-house method. The numbers of samples used for all crossmatch studies varied from 15 to 204 with equal variation between the numbers of males and females. Rebulla, Morelati et al. (2004) also noted that women were more refractory and could also bias studies that were using larger numbers of females than males due to more antibody production. Only a few studies included children and if others did they did not specify in their publication. Most of the patients used in the studies had haematology/oncology malignancies but a few had platelet disorders and thalassaemia which may have introduced too many variations. All studies should have ideally only included refractory patients due to immune or unknown factors, but many did not exclude patients with non-immune factors. The effect of these factors such as consumption of platelets by different mechanisms could influence the platelet counts after transfusion and introduce bias into the study. The number of variables should be controlled in a single study to ensure enough reliable information can be gained from it. Rebulla, Morelati et al. (2004) was the only study that mentioned how long the assays took and if this could possibly be implemented into a hospital laboratory. Many of the methods mentioned in the other studies would require specialist equipment and skilled staff to do the testing and would take hours to complete. There would be a preference for using automated devices with high throughput ability as this would generate important operational advantages for hospitals.

Gelb and Leavitt (1997) stated that 'they provided practical information on the usefulness of Capture-P in assisting the blood bank in providing these components at a tertiary care centre' but no evidence of this was in the actual paper.

An increased NPV indicates a greater chance of an adequate response to crossmatched platelets, whilst a high PPV indicates a lower number of possible compatible platelets incorrectly identified as incompatible. Only seven studies of the fifteen reported any PPV's or NPV's and only three of the study cohorts used Capture-P which is too small a number to rely on for accurate data. In general, the PPV's were all around 80% in those studies that reported them and a few more reported NPV's only This means that no one method is ideal, and compatibility identified with more than one technique may be better to predict successful platelet increments but is impractical and time consuming in a busy laboratory.

There were differing schools of thought on the order of crossmatching with Wiita and Nambiar (2012) using platelet crossmatching as a first line treatment with no increase rate of alloimmunisation seen, whereas the others tended to test for platelet antibodies first and then crossmatch HLA matched donors.

The most recent study by Revelli, Villa et al. (2019) reviewed the effectiveness of a platelet crossmatch programme for treating refractory haematology patients. This involved providing crossmatch compatible platelets first but once they became incompatible then HLA and HPA testing was done to provide typed platelets. This study was triggered by an investigation from Rioux-Masse, Cohn et al. (2014) who found that the use of crossmatch compatible or HLA matched units did not provide better increments in platelet count when compared to random units. As a result, they recommended that either or both HLA-matching and crossmatching be attempted for two transfusions and, if unsuccessful, regular

random donor units be used. This was the only study found where platelet increments did not increase after providing compatible platelets. Kieckbusch, et al., (1987) suggested that platelet crossmatching may be a useful additional study for predicting the outcome of transfusion – even in medically complex cases – if it can be done rapidly and relatively inexpensively. Bearing in mind that many refractory haematology patients have other underlying clinical conditions as well and are inherently associated with low platelet increments.

As mentioned, the limitation of all the crossmatch methods described was the need to perform frequent crossmatches for patients requiring ongoing transfusion support which can be labour intensive in a busy laboratory. A disadvantage of crossmatching is that crossmatched platelets have a short shelf life, so that crossmatch tests must be performed frequently for patients requiring long term platelet transfusions (Petz et al., 2000). The obvious advantage of crossmatching on available platelet donations is that a compatible transfusion could be available in a few hours rather than the several days it takes to schedule, draw, and test an HLA-typed donor. Both platelet crossmatching and HLA matched units were found to give similar success rates in most of the studies. However, the point raised was that crossmatching can only be successful if compatible units can be found and the length of time to find these needs to be considered.

HLA typed donors and patient crossmatching could be used together in selecting suitable donors for alloimmunised patients (Yung et al., 1991).

A cost analysis was only mentioned by Freedman, Gafni et al. (1989) and Rebulla, Morelati et al. (2004) even though certain assumptions were made in the former. Due to the number of crossmatches needed in some cases costings would be a necessary requirement and

should always be considered. Chakrabarty and Das (2017) and Pena, Sudhof et al. (2018) both used Capture-P crossmatch of platelets in patients with aplastic anaemia requiring surgery and a pregnant lady with aplastic anaemia, respectively. The success of the former case marked the beginning of issuing crossmatched platelets in Eastern India. The second case was the first reported case of managing platelet refractoriness in a pregnant patient with strong HLA antibodies in Boston. These represent individual case studies where platelet crossmatching has been used to provide platelets in a timely fashion according to patient need and have proved to be successful. Sayed, Bakry et al. (2011) and Salama, Aladl et al. (2014) both noticed that there was a poor response associated with platelets stored for more than 48 hrs which was interesting. None of the other researchers mentioned this. Most of the studies used a mixture of ABO, apheresis, and HLA matched platelets.

In conclusion there has been a lot of research on whether platelet crossmatching could increase platelet increments in haematology patients using many different methods, however, nothing consistent has led to an alteration of any guidelines. It has not yet been determined if crossmatching platelets could enable activated platelets to be detected in terms of their elevated levels of P-selectin, C3b and/or C1q and whether any incompatibility would be encountered in these cases.

Ideally the best method would be one that is fully automated, and this seems to be the solid phase red cell adherence assay. It also can include many platelet donations per plate giving the patient the best chance of finding compatible donations in a shorter time frame. There is also the possibility that platelet crossmatching could pick out patients that develop antibodies prior to testing for them. Also, the presence of activated platelets may elicit a

reaction with patient plasma that would result in them being substituted for a compatible platelet donation.

## 2.3.3 Acquired causes of platelet activation

### 2.3.3.1 Methods of platelet isolation

Most of the research that mentioned platelet preparation methods collected whole blood into sodium citrate tubes. Centrifugation speeds to prepare platelet rich plasma (PRP) varied from 140g to 700g for between 6 minutes to 20 minutes. Many studies mentioned fixing the platelets prior to testing to arrest any further activation that may occur during the processing steps, however, Wun, Paglieroni et al. (1992) mentioned that P-selectin antibodies did not bind as well after this as they found there was a decrease in the binding of monoclonal antibodies. Cahill, Macey et al. (1993) described a series of experiments comparing platelet activation antigen expression detected by flow cytometry in fixed and unfixed samples. Formaldehyde increased the expression of P-selectin and was not recommended when studying platelet activation. The concentrations of platelet rich plasma (PRP) used varied and would need to be tested with various techniques to see which concentration gave the best results. All platelet preparations were washed in a variety of buffers after their preparation, however, only one study (Saggu, Cortes et al. 2013) mentioned turning the brake off the centrifuge during this process to minimise artefactual platelet activation.

### 2.3.3.2 Platelet counts and mean platelet volume (MPV)

Most of the literature focusing on the success of platelet transfusions in raising the platelet count post-transfusion (platelet increments) uses the corrected count increment (CCI) calculation. This is calculated as follows:

Platelet increment x $10^9$/L × body surface area ($m^2$) /number of platelets transfused (× $10^{11}$) at 20 hours.

One unit of platelets transfused is taken as 3 x $10^{11}$/L which is the mean value for each donation. A successful post transfusion count is taken to be >10 X $10^9$/L or a CCI of 5000 (Slichter, Davis et al. 2005).

Literature reports were reviewed (Korniluk, Koper-Lenkiewicz et al. 2019) concerning changes in MPV and its possible role as a biomarker in various inflammatory processes and neoplastic diseases. They concluded that an increased MPV was observed in cardiovascular diseases, cerebral stroke, inflammatory disorders, and various cancers.

#### 2.3.3.3 P-Selectin and platelet activation

Several researchers (Fijnheer, Modderman et al. 1990)(Triulzi, Kickler et al. 1992, Wun, Paglieroni et al. 1992, Curvers, de Wildt-Eggen et al. 2008) have previously explored measuring the degree of platelet activation using a variety of markers, in particular P-selectin expression. Different methods have been used including agonist induced and naturally occurring platelet activation to assess platelet function with monoclonal antibodies and flow cytometry. A few studies have used dynamic light scattering to determine microparticle content with a ThromboLUX analyzer which determines whether platelets are activated or non-activated. Two groups of researchers (Bikker, Bouman et al. 2016) (Kicken, Roest et al. 2017) used agonist-induced P-selectin expression to see whether there was an adequate response after transfusion in patients with thrombocytopenia. The latter study mentioned that the assay used was time and cost-efficient. No other studies mentioned this and since many of these assays are time-consuming it would be an important factor to consider.

Many (Rinder, Murphy et al. 1991, Dunlop, Skinner et al. 1992, Holme, Sweeney et al. 1997, Dumont, AuBuchon et al. 2002) have tried to correlate the degree of platelet activation with post-transfusion platelet counts and different types of platelet donation and their storage including filtration and irradiation.

The data suggests that P-selectin exposure during storage triggers immediate P-selectin-mediated platelet concentrate clearance and it may compromise the quality of platelet donations as increased activation has been associated with decreased platelet recovery and survival in patients (Rinder, Murphy et al. 1991).

#### 2.3.3.4 Effect of platelet activation on platelet count

There has been conflicting data regarding the role of P-selectin and *in vivo* outcomes with respect to platelet counts and their recovery. Few (Holme, Sweeney et al. 1997, Dumont, AuBuchon et al. 2002) observed that the recovery and survival of autologous platelet transfusions to healthy volunteers correlate with P-selectin expression. It was observed (Triulzi, Kickler et al. 1992) that there was an inverse relationship between P-selectin expression and 1-hour CCI's in thrombocytopenic oncology patients, whereas Rinder, Murphy et al. (1991) were unable to show a significant inverse relationship (lower platelet counts correlate with higher percentage expression of P-selectin on platelets). This may be due to other immunologic/clinical factors. Wun, Paglieroni et al. (1992) studied plasmapheresis-induced platelet activation and concluded that activated platelets have short *in vivo* survival and found evidence of circulating activated platelets up to 48 hours after apheresis, without a fall in platelet count which contradicted the work by Rinder, Murphy et al. (1991). This study was extended to include testing of donors prior to collection of platelet donations by apheresis and if platelet activation were abnormal the

collection procedure would be delayed. Putting this into practice in donation centres would be costly and possibly delay platelet preparation as well as rescheduling of the donor. Bock, Rahrig et al. (2002) also questioned whether *in vivo* studies should be performed to confirm the results of any *in vitro* results to see if they have clinical impact or are they just simply observations. The study by Leytin, Allen et al. (2004) revealed that P-selectin expression correlated only with rapid post-transfusion clearance of platelets but not with delayed platelet removal. Strindberg and Berlin (1996) could not find any difference between platelet concentrates and apheresis platelets in post-transfusion-corrected count increments. Furthermore, it has been demonstrated that activation markers are lost from the surface of transfused platelets as they circulate, and that platelet activation does not apparently induce loss of *in vivo* viability (Michelson, Barnard et al. 1996).

Both Holme, Sweeney et al. (1997) and Bikker, Bouman et al. (2016) agreed that most P-selectin-positive platelets are most likely cleared from the circulation upon transfusion and can be a predictor of platelet viability. This suggests that prevention of platelet activation should be an important target of future research on the improvement of platelet quality during storage. Arnold, Heddle et al. (2006) looked at *in vivo* recovery and survival of apheresis and whole blood-derived platelets in healthy volunteers. They found that the whole blood-derived platelets appeared to adversely affect platelet recovery and survival. Saris, Kreuger et al. (2019) also used a platelet responsiveness assay and stated that storage of platelet concentrates result in reduced recovery and survival of transfused platelets. This study also concluded that patient-related factors are the main determinants of the effectivity of platelet transfusions and CCI's may not be ideal to monitor the clinical outcome after a transfusion.

#### 2.3.3.5 Soluble P-selectin and other markers

Palabrica, Furie et al. (1989) states that P-selectin is not exposed on either circulating resting platelets nor present in a soluble form in plasma. Whereas Michelson, Barnard et al. (1996) concluded that methods that evaluate soluble P-selectin may be of more value clinically than platelet surface P-selectin expression for evaluating circulation of degranulated platelets. Divers, Kannan et al. (1995) found a gradual increase in P-selectin levels in the supernatant suggesting that this is secreted from platelets as they become activated during storage. The measurement of soluble P-selectin also correlated closely with platelet derived particles formation in stored platelet concentrates and can also provide an assessment of platelet activation and quality. Dunlop, Skinner et al. (1992) supported the fact that P-selectin release was activation dependent.

Skripchenko, Kurtz et al. (2008) compared surface levels of CD40L and P-selectin and accumulation of the soluble forms of these activation markers in the plasma of stored platelets prepared from pooled platelets and two apheresis instruments. Both CD40L and P-selectin are expressed on platelets and subsequently secreted into the plasma by proteolytic cleavage to represent a soluble form. Levels of all markers were increased in all platelet preparations – however, they were highest in apheresis platelets (Skripchenko, Kurtz et al. 2008, Bikker, Bouman et al. 2016). Sut, Tariket et al. (2019) agreed that platelet activation was lower in pooled platelets when comparing the same markers.

During the collection of apheresis platelets Bakry, Sayed et al. (2010) found that levels of both CD40L and P-selectin increased during storage. This could be explained by the manner of agitation, pH changes, numbers of white blood cells or exposure to surfaces and forces during the apheresis procedure. Whilst Holme, Sweeney et al. (1997) found that P-selectin

expression on the platelet surface is a predictor of platelet viability, the extent of shape change, and hypotonic shock were more sensitive predictors.

Curvers, de Wildt-Eggen et al. (2008) tried to standardise a method to measure P-selectin on platelets between different centres and got comparable results – however, centres need to establish their own methods and normal range.

#### 2.3.3.6 Different types of platelet donations

The platelets used in this research were a mixture of pooled and apheresis donations, however, they were all leucodepleted and irradiated according to guidelines for haematology patients (Foukaneli, Kerr et al. 2020). Previous research supported the fact that effects of irradiation either before or after storage were minimal (Moroff, George et al. 1986, Rock, Adams et al. 1988, Tynngard, Studer et al. 2008). Expression of P-selectin on platelets has been used as a measure of quality of buffy coat and apheresis platelet donations by flow cytometry (Bock, Rahrig et al. 2002) and this correlates with loss of viability upon transfusion of the stored platelet concentrates (Bakry, Sayed et al. 2010).

Holme, Sweeney et al. (1997) found that different apheresis machines yield different results in terms of the extent of P-selectin expression during the collection, processing, and storage of platelet concentrates.

Several researchers found that activation indicated by P-selectin expression consistently increased over the storage period for platelet donations after testing over a series of days prior to their expiry. Fijnheer, Modderman et al. (1990) showed clear platelet activation during storage due to increased levels of all markers used. The data suggests that P-selectin exposure during storage triggers immediate P-selectin-mediated platelet concentrate clearance and it may compromise the quality of platelet donations as increased activation

has been associated with decreased platelet recovery and survival in patients (Rinder, Murphy et al. 1991).

Pedigo, Wun et al. (1993) found no significant increases in platelet activation markers after leucodepletion. Any decrease in platelet count tended to correlate with increased numbers of activated platelets which would explain any platelet loss in some components. Triulzi, Kickler et al. (1992) agreed that activation was not increased by filtration but did not find that activated platelets were removed.

### 2.3.4 Effect of complement on platelets

There were many studies looking at complement proteins in stored platelet concentrates. Chen, Losos et al. (2017) demonstrated that substantial complement activation occurs in platelets under standard storage conditions, and this activation increased with duration of storage. It was also suggested that after transfusion of these platelets accelerated complement activation could lead to transfusion-related adverse events.

Gyongyossy-Issa, McLeod et al. (1994) investigated the role of complement activation as a possible mediator of the platelet storage lesion. They studied the generation of several complement components and found that levels of soluble C4d and C3a increased steadily over time in storage, as did the level of the inactivated membrane attack complex SC5b-9. They detected C3 on the surface of platelets and the percentage of these peaked at day 3 of storage and then fell again.

Del Conde, Cruz et al. (2005) found that platelets can activate the complement system and identified P-selectin as a receptor for C3b which is capable of initiating complement activation via the alternative pathway thus linking thrombosis and local activation of the

complement system on platelets. Next Peerschke, Yin et al. (2006) provided evidence for C1q dependent classical complement pathway activation on platelets and supported a role for gC1qR (globular C1q receptor) in this process. Together these were starting to support a growing body of evidence regarding the role of platelets and complement in inflammatory conditions and vascular injury. These receptors and their capacity to activate the different complement pathways are shown in Figure 2.1.

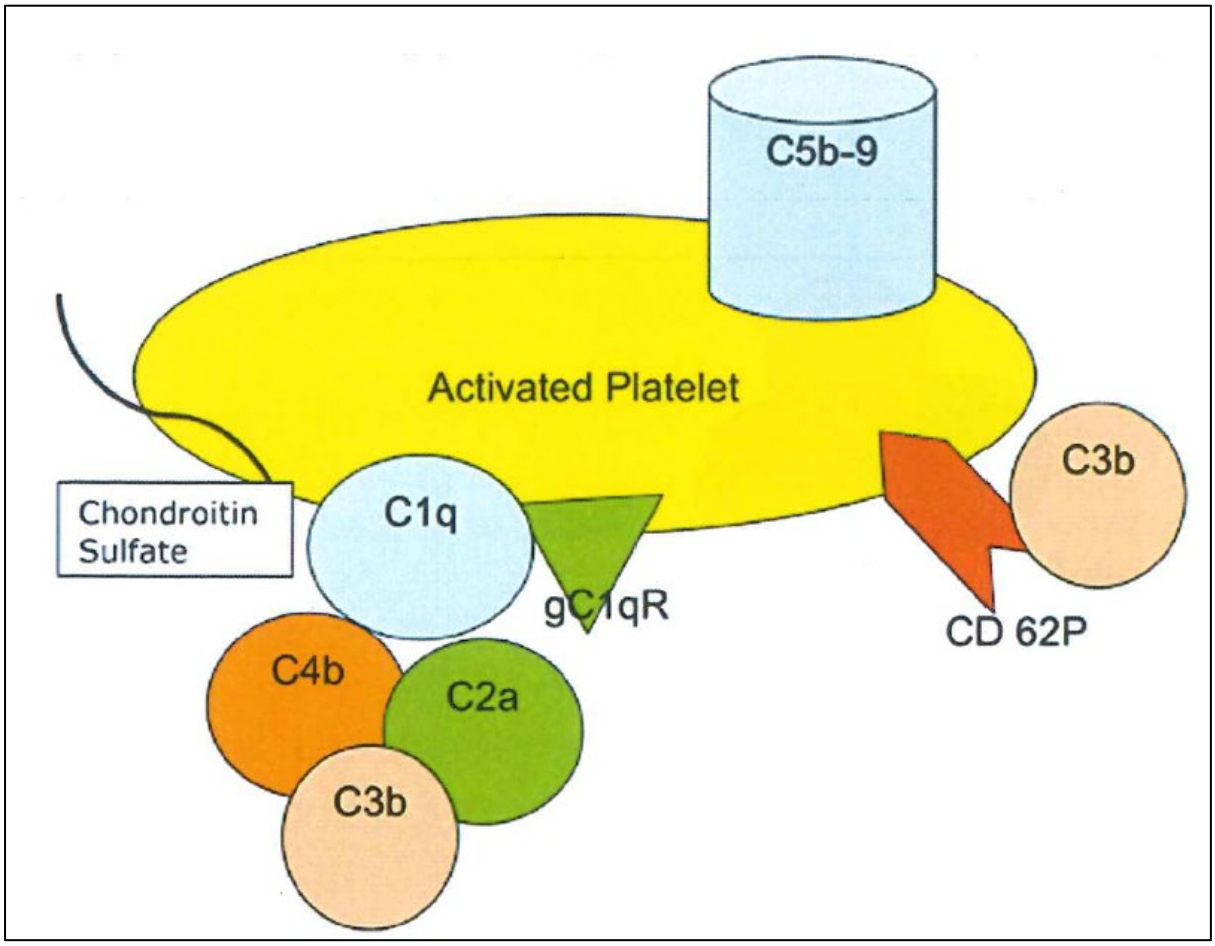


*Figure 2.1: Proposed mechanisms of complement activation on/by activated platelets and the intrinsic capacity of platelets to activate the classical and alternative pathways of complement (Taken from Peerschke et al., 2010), showing the different components and their assembly.*

This provided evidence that activated platelets have binding sites for complement components C3b and C1q which can trigger a variety of biochemical and cellular responses that may contribute to inflammation and thrombosis because of premature platelet activation (Peerschke, Yin et al. 2006, Peerschke, Yin et al. 2008, Peerschke, Yin et al. 2010).

However, Hamad, Nilsson et al. (2010) disagreed with the fact that complement was activated on the platelet surface due to the large number of complement regulators found on the activated platelet surface that probably serve to protect the platelets from complement attack. They concluded that under physiological conditions no complement activation occurs on the surfaces of platelets else there would be evidence of platelet damage and thrombotic reactions. In a few pathological conditions in which regulators of complement activation are lacking e.g., PNH (paroxysmal nocturnal haemoglobinuria) this is detrimental to platelets and leads to platelet activation and thrombotic complications (Del Conde, Cruz et al. 2005).

Skoglund, Wettero et al. (2010) demonstrated that C1q caused a moderate up-regulation of P-selectin on the platelet surface which seemed to be initiated by C1q binding to gC1qR (receptor for C1q). The increase in P-selectin expression upon C1q stimulation observed in this study could be sufficient to trigger further activation of complement, without leading to massive platelet activation and this could play an important role at sites of inflammation.

Peerschke, Yin et al. (2008) proposed that platelets concentrate complement activation to sites of vascular injury, and that interactions between platelets and the complement system contribute to acute and chronic inflammation and thrombosis.

Complement activation could occur undetected in a platelet donor and when transfused to haematology patients their immune system may become overwhelmed by these activated platelets. Maurer-Spurej, Labrie et al. (2009) used a ThromboLUX analyser which measures the pattern of laser light scattered by platelets and platelet derived particles (microparticles) to determine their size when exposed to different temperature conditions and this can be used to definitively characterize each platelet bag as activated or non-activated. In this pilot study, the ThromboLUX analyser score strongly correlated with transfusion outcome (platelet recovery and survival) independent of clinical and product issues. When transfusing only the non-activated donations to the haematology-oncology patients they noticed a 17% reduction in platelet demand for these patients as a result – with a total of 89 transfusions saved over 4 months (Maurer-Spurej 2018). Labrie (2018) suggested that activated platelets become opsonised by circulating complement which results in clearance of platelets by macrophages over 6 to 24 hours instead of the immediate (1 hour) clearance associated with antibody formation (Figure 2.2).

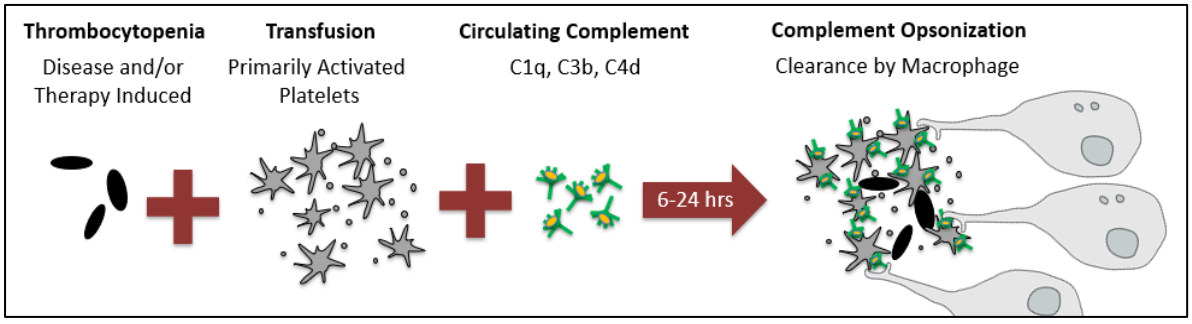


*Figure 2.2: Proposed process of complement mediated platelet removal after transfusion (Labrie 2018) showing how activated platelets can become opsonised by complement components C1q, C3b and C4d and removed by macrophages.*

Various proteins of the complement system interact with platelets, which can lead to their activation. Activated platelets become opsonised by circulating complement, namely C1q

and C3b which enables macrophages to recognise them and remove them by phagocytosis. P-selectin once expressed on the platelet surface has been found to be a receptor for C3b and a receptor for C1q is also present on activated platelets and these are known triggers for complement activation which could also have a role to play in the activation and subsequent removal of platelets by macrophages. Measuring levels of C3 and C1q on platelets could be an indication of whether complement activation has occurred.

Rijkers, Schmidt et al. (2019) proposed that if HLA antibodies exist, they will bind to HLA molecules on the platelets. C1q can bind to HLA bound IgG's leading to initiation of the classical complement pathway and C3b deposition on the platelet surface. Fontaine, Kuo et al. (2011) also found that HLA antibodies binding to C1q were more clinically significant than those binding to IgG as they are more capable of activating the complement cascade causing platelet destruction.

The effect of complement on platelets has been investigated in certain haematological conditions where it has been linked to reduced platelet survival and venous thrombosis as mentioned in the introduction (Myers, Kim et al. 1982, Lood, Eriksson et al. 2012).

## 2.4 Discussion of results

Platelet crossmatching research has been taking place for over 40 years now using a variety of different methods, but despite the time and effort involved in obtaining platelets from apheresis donors and the risk of bleeding in patients not provided with compatible platelets, platelet crossmatching has never taken off in the UK. Also, the research seems to consist of similar repetitive studies where platelet count data is collected pre and post transfusion in

patients with differing haematological conditions, platelets are crossmatched with the majority using the solid phase method and there does not seem to be any progress made.

Several broad observations can be made from this analysis:

- Platelet crossmatching in the UK is not likely to become part of a national guideline anytime soon
- There is conflicting evidence in terms of correlation of platelet count increment with a compatible platelet crossmatch using different methods
- P-selectin expression is the most researched platelet activation marker
- Apheresis platelet donations have more expression of P-selectin due to contact with artificial surfaces during extracorporeal circulation procedures
- There is an increase in platelet activation with storage age of platelet donations
- Increased complement activation occurs during platelet storage
- There is a distinct lack of research into the effect of complement on platelet activation
- There are no published papers on the effect of complement present on platelets and the effect this may have on a platelet crossmatch result

Many different methods for crossmatching of platelets have been seen in the articles reviewed with some of them taking many hours to perform before any results are seen. There has also been conflicting data on the efficiency of these methods in terms of sensitivity and specificity. If platelet crossmatching were to be done successfully the method would be suited for automation on a walkaway analyzer rather than manual technology and most studies did use the solid phase technology. This would also ensure consistency between laboratories and maximizing the potential of any equipment purchased. Another

drawback with crossmatching platelets was the reduced availability of stock, short expiry dates and stock holding areas. Few hospitals have used platelet crossmatching for individual patients and this tends to be in emergency situations where there is no increment, and a patient requires surgery and, in some cases, crossmatching has been used in conjunction with HLA/HPA matched platelets.

The increased level of platelet activation seen in platelet donations during their storage time prior to expiry is a consistent finding in many articles. This is important as activated platelets tend to be preferentially cleared from the circulation reducing the platelet count more quickly than intended. There is conflicting evidence over the type of donations which appear to have the most activated platelets in with the apheresis technology being blamed for their increased platelet activation. All platelet donations require some processing to enable the donation to be fit for purpose and in general this will cause some platelet activation due to their sensitive nature and need to be handled gently. Transfusing activated platelets into patients can cancel the advantage they were meant to have and maybe introducing viability assays to detect platelet activation in donations prior to transfusion could be important for the future.

Limitations of this article review was the variation in technology used for platelet crossmatching and measurement of platelet function and the use of different markers of platelet activation which made any comparisons difficult.

## 2.5 Conclusion

This review highlights the fact that more research is required on platelet crossmatching to understand the reasons why some crossmatches are incompatible in the absence of HLA/HPA antibodies and non-immune mechanisms. There has been some work undertaken

with complement in relation to the fact that complement activation can occur on platelets but not on the effects that this could have on the platelet crossmatching procedure.

The data also highlights the fact that the process of donating apheresis platelets and processing of buffy coats for platelet donations can increase platelet and complement activation which can have a deleterious effect on the platelet increment of the patient post transfusion.

It is now becoming more evident how complicated platelets are in terms of their structure and functions and how challenging future research will be.

# Chapter 3 Materials and Methods

## 3.1 Ethics Approval

This study has full Health Research Authority (HRA) approval and Health and Care Research Wales (HCRW) approval under the Integrated Research Application System (IRAS). IRAS project ID: 248801 (Appendix B).

## 3.2 Data Collection

The project involved recruitment within the laboratory of twenty normal volunteers (10 male and 10 female) from which to establish a reference range for the markers CD62P, C1q, C3, IgG and C3dg as there are currently no published ranges for these.

To determine the effect of platelet transfusions on these markers in haematology patients, 8 patients were tested with a total of 92 platelet transfusion episodes amongst them. Haematology patients used in this study were those who had allogeneic HSCT's and those on chemotherapy for an underlying haematological malignancy. The consented patients were allocated a patient number to anonymise the data. The age range was 63 ± 10 years with the majority being male. There were 2 patients with the most transfusions episodes between them until one developed HLA antibodies and the other was no longer refractory. A total of 2 patients developed HLA antibodies and were removed from the study and 2 died. Five patients became refractory after allogeneic stem cell transplants and the rest were because of palliative chemotherapy. The haematology patient demographics are summarised in Table A2 in the Appendix.

### 3.2.1 Informed Consent and Confidentiality

All contact with patients was conducted solely through a transfusion practitioner nurse who approached the available haematology patients through the day case or haematology ward on level 8 University Hospital Plymouth (UHP), UK. The subjects were given an information sheet and a signed consent form was obtained prior to sampling (Appendix C). All informed consent paperwork was kept in the Blood Transfusion Laboratory and transferred to UHP research offices at the conclusion of the study. Laboratory staff did not have access to this data at any time and the only possible identifier was a number that did not relate to any of their information and was a patient number to anonymise the data.

## 3.3 Sample/Platelet Preparation and Storage

Peripheral blood from consented adults was drawn into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant as this prevents further complement activation. These were centrifuged at 200*g* with no brake using a Heraeus Megafuge 8 from Thermo Fisher Scientific, Waltham, Massachusetts, United States of America (USA) for 10 minutes to get platelet rich plasma (PRP) (Peerschke, Yin et al. 2006). An aliquot of the platelet suspension was removed from the donor bags and transferred to a plastic test tube to serve as the PRP. Washing and storing of platelets derived from PRP must be performed within 48 hours after specimen collection. Platelets obtained from platelet components must be used within the expiration dates of the units and washed and suspended within 24 hours of the collection date and time. The PRP was then centrifuged for 5 minutes at 1000*g,* and the PPP removed from the platelet pellet. Platelet Wash and Storage Solution (PWSS) from Immucor, Georgia, USA, was added to each sample and the

platelets gently re-suspended on the platelet agitator. PWSS is used to partially replace plasma in the preparation and storage of buffy coat-derived platelet concentrates or apheresis platelet units. It typically includes several compounds (sodium chloride, sodium citrate, potassium chloride and magnesium chloride) to maintain optimal pH and minimize platelet aggregation at room temperature. It is supplied in a polypropylene bag with a giving set. Any samples derived from platelet components must be diluted to within the acceptable range using PWSS. The final platelet concentration should be between 20,000/µl and 350,000/µl therefore depending on what the original platelet count will dictate the amount of PWSS required for dilution. Any platelets that have been washed and suspended in PWSS can be used immediately or stored at 1-10$^{o}$C for up to eight months prior to use.

### 3.3.1 Platelet Count

Platelet counts and MPV's were analysed on samples from patients prior to platelet transfusion and up to 12 hours post transfusion. This was done using the Alinity $^{TM}$ h-series integrated haematology analyser from Abbott Diagnostics, Chicago, Illinois, USA. This analyser delivers a complete blood count (CBC) with a 6-part white blood cell (WBC) differential and several parameters using advanced MAPSS (Multi Angle Polarized Scatter Separation) technology. The design incorporates additional angular intervals of light scatter to improve differentiation of the WBC, red blood cells (RBC) and platelets as they pass through the detection area of the optical flow cell.

All platelet donations issued and transfused to patients were ABO compatible and if not were always high titre negative (titres of anti-A and anti-B antibodies are <100 at room temperature). All patients were haematology patients and therefore a successful post

transfusion count would be >10 X $10^9$/L or a CCI of 5000 (Slichter, Davis et al. 2005) using the formula:

Platelet increment x $10^9$/L × body surface area in square meters / number of platelets transfused (× $10^{11}$) at 20 hours.

One unit of platelets transfused was taken as 3 x $10^{11}$/L which is the mean value for each donation.

## 3.4 Flow Cytometric Analysis

Immediately after staining the samples were analysed using the FACSLyric flow cytometer from Becton Dickinson, Oxford, United Kingdom (UK). The flow cytometer was setup according to the manufacturer's instructions using BD™ CS&T beads from Becton Dickinson. These beads are a suspension of fluorospheres with uniform and stable size and fluorescence intensity and are used for instrument quality control to characterise, track and report performance measurements of the flow cytometer. The forward and side scatter were set at logarithmic amplification with the platelets centred in the plot between $10^3$ and $10^5$. Platelets were identified by their specific light scattering characteristics using CD61 (platelet glycoprotein IIIa) with a gate set around this population and a total of 10,000 events being collected and analysed (Figure 3.1).

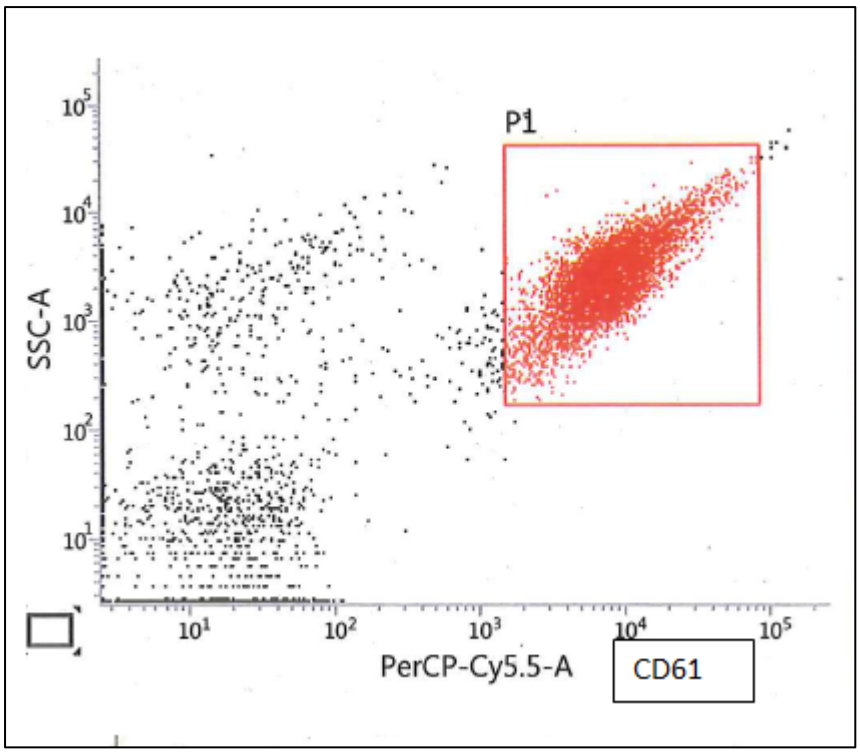


*Figure 3.1: This shows the gating of the platelets. The forward and side scatter were set at logarithmic amplification with the platelets centred in the plot between $10^3$ and $10^5$. Platelets were identified by their specific light scattering characteristics using CD61 (platelet glycoprotein IIIa) with a gate set around this population and a total of 10,000 events being collected and analysed on the FACSLyric flow cytometer from Becton Dickinson, Oxford, United Kingdom (UK).*

### 3.4.1 Determination of optimal antibody concentrations

The optimal reactions between platelets and different concentrations of antisera were determined prior to testing and this was 5µl of antibody per 50µl sample of $10^9$ platelets. This was determined by using amounts of antibody as recommended by the manufacturers, but also testing dilutions either side of these. The stain index calculation was also checked to see if the results were the same. The stain index calculation is the median fluorescence intensity (MFI) of the positive sample – MFI of the negative sample all divided by 2 X the standard deviation of the negative population. The antibodies chosen were already conjugated and mostly sold for research purposes only. The problems encountered included

difficulty in sourcing due to them not being used routinely and the lack of controls for the assays. Controls had to be made using sensitised red cells, controls from Immunology and platelets activated with ADP to ensure that the antibodies worked. External quality assessment of such assays would be difficult and reproducibility therefore questionable. The concentrations of antibodies recommended for use by the manufacturers were generous and smaller quantities were adequate for the flow cytometry testing and these are shown in Table 3.2. Isotypic controls (Table 3.1) were used to indicate the negative populations for each marker and any platelet events with fluorescence intensity higher than this were considered positive.

*Table 3.1: List of isotypic controls used in this study*

| **Isotypic Control** | **Manufacturer** | **Volume of isotype antibody added** |
|---|---|---|
| Mouse IgG conjugated to peridinin chlorophyll protein complex | Catalogue number MPERCPCON from Bioquote Limited, York, UK | 5µl |
| Rabbit IgG conjugated to fluorescein isothiocyanate | Catalogue number ab37406 from Abcam, Cambridge, UK | 5µl |
| Mouse IgG1 conjugated to brilliant violet | Catalogue number 562438 from BD, Oxford, UK | 5µl |
| Mouse IgG1 conjugated to Allophycocyanin | Catalogue number 555751 from BD, Oxford, UK | 5µl |

*Table 3.2: List of conjugated antibodies used in this study*

| **Reagent** | **Manufacturer** | **Target** | **Volume of antibody added** |
|---|---|---|---|
| Rabbit polyclonal to human C1q conjugated to fluorescein isothiocyanate | catalogue number ab4223 from Abcam, Cambridge, UK | Complement C1q | 5µl |
| Human Complement C3 monoclonal antibody conjugated to peridinin chlorophyll protein complex | Catalogue number 60231-05071 from Bioquote Limited, York, UK | Complement C3 | 5µl |
| Human Complement C3dg conjugated to fluorescein isothiocyanate | Catalogue number 11294-05041 from Bioquote Limited, York, UK | Complement C3dg | 5µl |
| Human IgG conjugated to Allophycocyanin | Catalogue number 550931 from Becton Dickinson (BD), Oxford, UK | IgG | 5µl |
| CD62P conjugated to brilliant violet | Catalogue number 564038 from BD, Oxford, UK | P-Selectin | 5µl |
| CD61 conjugated to brilliant violet | Catalogue number 564038 from BD, Oxford, UK | Platelet glycoprotein IIIa | 5µl |
| CD61 conjugated to peridinin chlorophyll protein complex cyanine dye | Catalogue number 564173 from BD, Oxford, UK | Platelet glycoprotein IIIa | 5µl |

Once staining was complete the platelets were washed 4 times and re-suspended with 50µl of PWSS prior to flow cytometry.

### 3.4.2 Flow Cytometric Determination of CD62P, Complement and IgG

Flow cytometry was used to demonstrate binding of complement proteins and activation of platelets. A 50µl volume of a $10^9$ sample of platelets was incubated for 60 min at RT (room temperature) in the dark with already conjugated antibodies (Table 3.2).

The platelet population within each sample was stained with a variety of conjugated markers for IgG, C3b, C1q, C3dg and CD62P. The results of these are shown in histograms that are single parameter plots that give information on the percentage and number of cells positive for the markers (Figure 3.2) with the isotypic controls taken as the negative populations. The data is expressed in a histogram as it represents a selected (gated) population of platelets which express the markers. The median fluorescence intensity (MFI) value was used to compare expressions of the target of interest across samples, which included IgG, C3b, C1q, C3dg and CD62P.

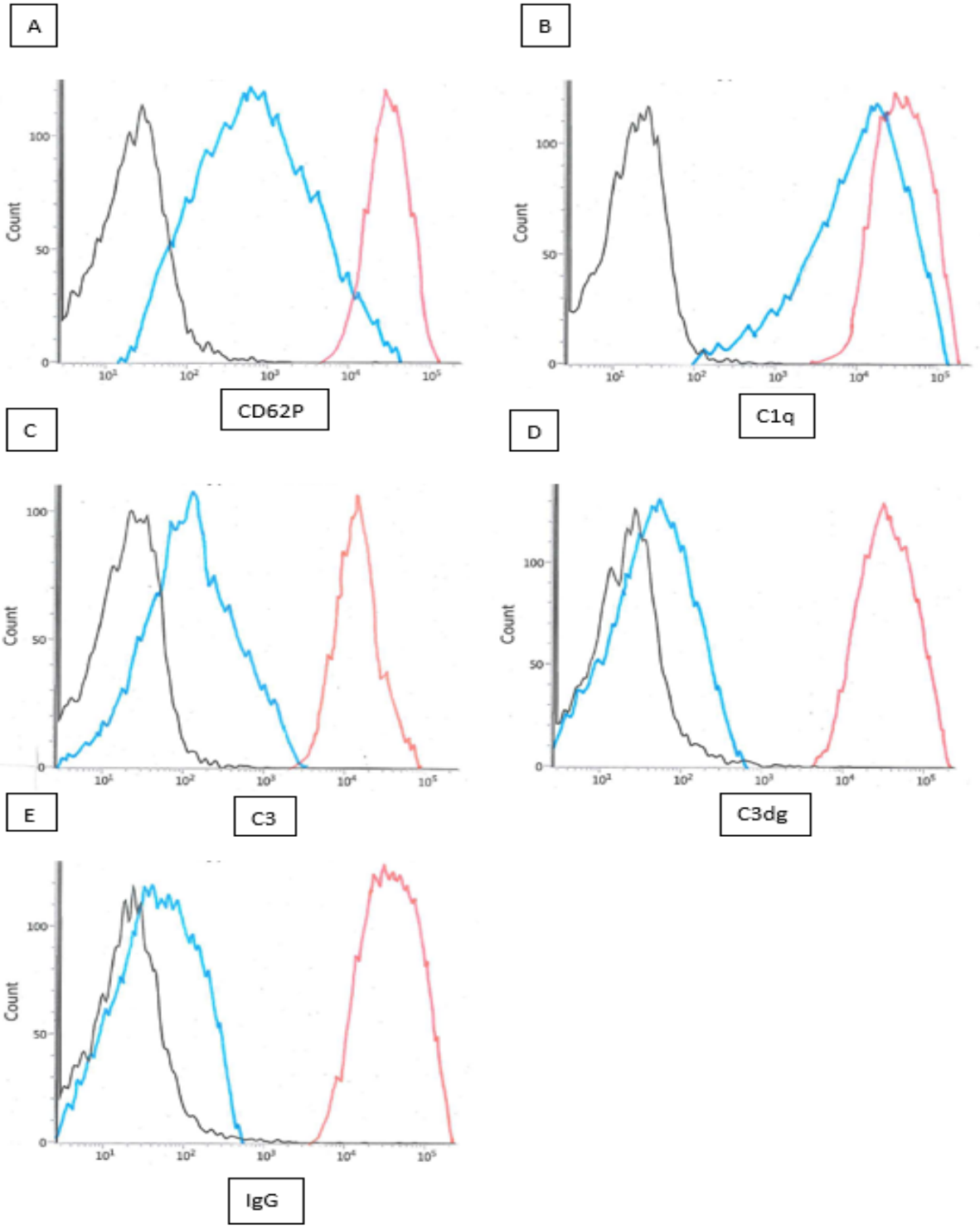


*Figure 3.2: Histograms to show the binding of platelet markers anti-CD62P (A), anti-C1q (B), anti-C3 (C), anti-C3dg (D) and anti-IgG (E) to samples of platelets with varying degrees of positivity (blue line) as detected by flow cytometry. The histograms represent all the gated platelets that express the marker of interest on the x-axis and the number of events (cell count) on the y-axis. The black lines represent the isotype antibody controls (lack specificity for the target antigen) and the red lines the in-house positive controls. This illustrates that the target antigen is expressed on the relevant cells.*

## 3.5 Platelet Crossmatch Assays

Serological platelet crossmatch was carried out on the NEO Iris™ which is the 6th fully automated blood bank analyser from Immucor, Georgia, USA, on which the assay had been installed using the manufacturer's instructions (This detection system had been modified from procedures published by Rachel, Sinor et al. (1985), Juji, Kano et al. (1972) and Shibata, Juji et al. (1981). The Capture P crossmatch plates uses the principles of solid phase technology and are coated with a specific platelet binding agent and stored in a resealable foil pouch to which a dessicant and moisture indicator have been added. Platelets are washed free of contaminating plasma proteins and non-platelet cellular elements with PWSS and then can be used to prepare monolayers of platelets from donors in Capture P test wells. Patient or donor platelets can be bound to the surfaces of the plate wells and subsequently be used to capture platelet antibodies. The concentration of platelets used to prepare the platelet monolayers can affect the outcome of platelet antibody detection tests. When the platelet suspension contains too few platelets (count below 10,000/$mm^3$) incomplete monolayers may be formed and lead to false positive results. If the count of the platelet suspension is too high (>350,000/$mm^3$) false negative results can occur. 100µl of platelet suspension is added to each well of the Capture P crossmatch plates and this is centrifuged at 45-65*g* for 5 minutes. Excess unbound platelets and plasma is removed using the fully automated washing step. Platelets are sticky by nature and when too many are present, they adhere to one another during the centrifugation step to prepare the monolayer. They are not all removed during the first washing step and therefore, a second layer is left on top of the primary monolayer. However, the second layer is not as firmly bound as the primary monolayer that is attached to the coupling agent at the well bottom.

Next 100µl of LISS (low ionic strength saline) is added and this will prevent any drying of the wells and possible disruption of the platelet monolayer. The LISS is also intended for use as a potentiator which in lowering the concentration of salt increases the rate of specific antibody uptake. After addition of LISS 50µl of the patient samples are added and 50µl of the negative and positive control samples into the relevant wells. These are incubated in platelet coated wells at 39$^{o}$C for 40 minutes to allow antibodies, if present, to bind to the platelets. Unbound immunoglobulins are then washed from the wells and 60µl of a suspension of anti-IgG coated indicator red cells are added. Centrifugation at 700-900*g* for a minute brings the indicator cells in contact with any antibodies bound to the immobilised platelets. In a positive test the anti-IgG forms bridges between the indicator cells and the platelet bound antibodies resulting in the formation of a monolayer. Varying degrees of positivity can be seen from an incomplete to a full monolayer. In the absence of antibodies, the indicator cells will pellet at the bottom of the wells to form a button and a negative test. The reactivity of the Capture-P test system is evaluated at each use by inclusion of positive and negative control tests.

An example of the results obtained from a crossmatch using Capture-P can be seen in Figure 3.3. The difference between a compatible and an incompatible unit are quite clearly seen, however, the analyser will read these results giving the compatible units a negative result and the incompatible ones a positive result enabling the process to be fully automated.

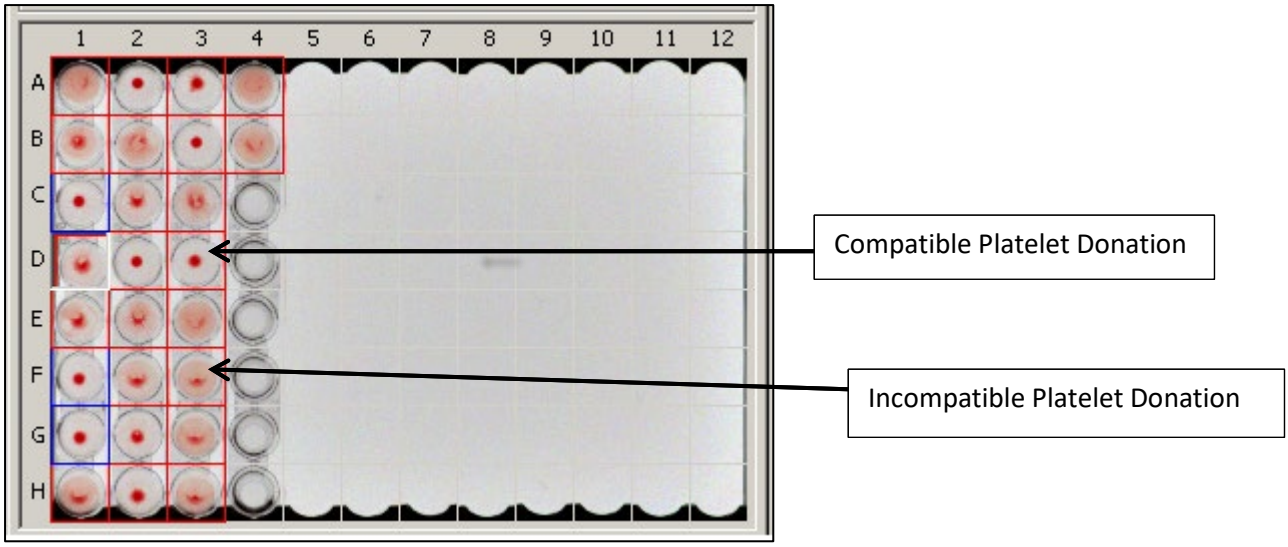


*Figure 3.3: This shows a microtitre plate which illustrates the results for compatible and incompatible platelet donations when crossmatching units of platelets using the Capture-P technology on the NEO Iris™ which is the 6th fully automated blood bank analyser from Immucor, Georgia, USA. A compatible platelet donation consists of a single button of cells in the centre of the well, whilst an incompatible donation shows varying degrees of positivity in the well.*

## 3.6 Statistical/Data Analysis

All data sets obtained were tested for normality using the Kolmogorov-Smirnov normality test using Graphpad PRISM, version 9.4.0.673. Any data found to be non-parametric was represented using median and range and evaluated statistically by the One-way ANOVA and Spearman's rank correlation coefficient. Data found to be parametric was represented using the mean and standard deviation and evaluated statistically by the t-test and Pearson's rank correlation coefficient.

# Chapter 4 Results

## 4.1 Introduction

The aim of this study was to see if haematology patients had improved platelet increments after transfusions of crossmatch compatible platelets. The primary objective was measurement of platelet counts in haematology patients to determine whether providing crossmatch compatible platelets increased the platelet count more readily than providing random donor platelets. In the cases where incompatible platelet transfusions were encountered the possibility of activation within the platelet donations and resultant platelet destruction was thought to be a possible cause. Therefore, the testing was extended to looking at markers on the platelets in apheresis, pooled platelet donations and haematology patients to see if any of these could account for the refractoriness. These were specifically IgG (to ensure no platelet antibody was present), CD62P (a platelet activation marker for P-selectin), MPV (a possible sign of platelet activation due to shape change) and complement proteins (C1q, C3 and C3dg) which opsonise activated platelets and result in their clearance by macrophages over 6 to 24 hours. The levels of these markers were compared with crossmatch results and transfusion outcomes.

## 4.2 Crossmatch compatible platelets do not improve platelet increments in haematology patients

The platelet crossmatch results for each patient were put into tables (Table A4-11 in Appendix A) showing the platelet characteristics, the crossmatch results and whether the transfusions produced a satisfactory increment or not. A transfusion was deemed to be successful where the CCI was >5000 or the platelet count went over 10 X $10^9$/L. The

crossmatch results and whether they led to successful or unsuccessful platelet transfusions are represented in Figure 4.1. A successful transfusion could not be attributed to the provision of compatible platelets, since the percentage of successful transfusions were similar between the 'compatible' and 'incompatible' transfusion groups. The results illustrate that providing crossmatch compatible platelets does not improve the platelet increments in the haematology patients in this study.

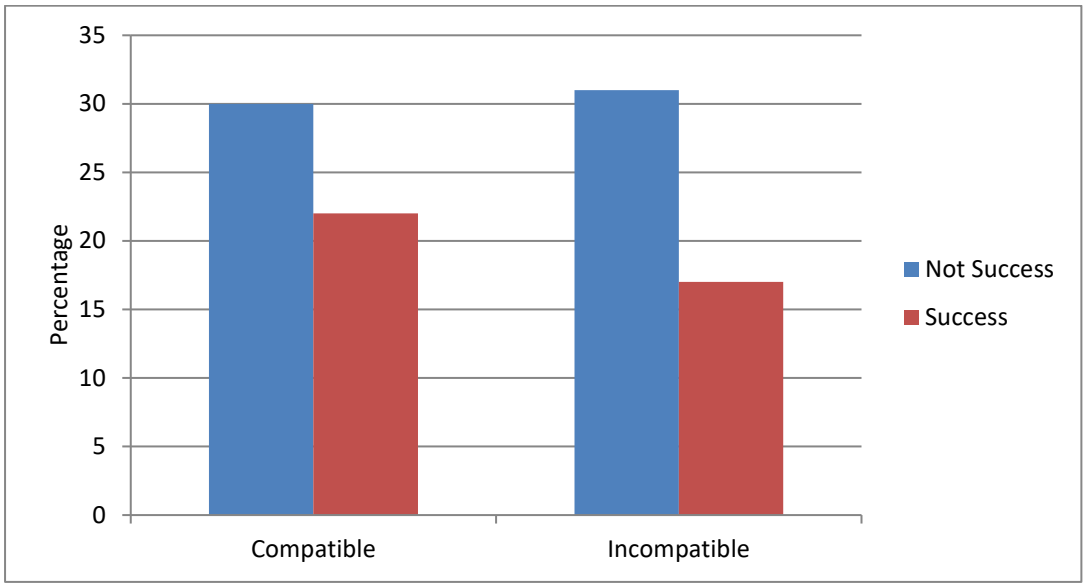


*Figure 4.1: This shows the percentage of compatible and incompatible platelet transfusions and whether they were successful (in red) or unsuccessful (in blue). A transfusion was deemed to be successful where the CCI was >5000 or the platelet count went over 10 X $10^9$/L.*

### 4.2.1 Calculations of sensitivity, specificity, and efficiency of the Capture-P crossmatch method

The Capture-P method used for crossmatching is not used routinely and therefore it was useful to analyse the value of this method according to sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and total predictive (TPV) value using predictive value analysis. True positive (TP) is the number of transfusion failures (below

expected CCI) following use of incompatible unit(s). True negative (TN) is the number of successful transfusions following use of compatible unit(s). False negative (FN) is the number of transfusion failures following use of compatible unit(s). False positive (FP) is the number of successful transfusions following use of incompatible units. The results from the predictive value analysis can be seen in Table 4.1.

*Table 4.1: Predictive value analysis of the Capture-P method*

| Predictive Value Analysis | Calculation | Result from this study |
|---|---|---|
| Sensitivity | TP / TP + FN x 100% | 44% |
| Specificity | TN / TN + FP x 100% | 49% |
| Predictive value of a positive test result (% of positive results that are transfusion failures) | TP / TP + FP x 100% | 36% |
| Predictive value of a negative test result (% of negative results that are transfusion successes). | TN / FN + TN x 100% | 57% |
| Efficiency (% of all results that are true results, whether positive or negative) | TP + TN / TP + TN + FP + FN x 100% | 47% |

These results show quite clearly that the Capture-P crossmatch method in this study was only 47% efficient, with both specificity and sensitivity being <50%.

## 4.3 Apheresis platelet donations and haematology patients have raised levels of CD62P and C1q

The results showed that transfusion of a compatible platelet donation did not increase the platelet increments in haematology patients. This lack of successful transfusion would usually be attributed to immune-mediated mechanisms – however, these were thought to be eliminated by testing for the presence of platelet antibodies. Therefore, markers of complement activation and P-selectin as a marker of activated platelets were measured to see if any of these could account for the unsuccessful transfusion outcomes. Anti-IgG was included as a test marker to ensure that continual monitoring of immune mechanisms was maintained.

### 4.3.1 Calculation of Normal Ranges

There are no documented normal ranges for comparison for IgG, C3b, C1q, C3dg and CD62P, therefore, a mixture of 10 normal males and 10 normal females with no known medical conditions were tested to establish normal ranges for all markers being used in this study as shown in Table A12 in Appendix A. These normal ranges were required as a baseline to enable comparison of the markers in haematology patients compared to normal controls. The data obtained from analysis of these platelet markers in the 20 normal patients were analysed for normality using the Kolmogorov-Smirnov test and found to all follow normal distributions (the results from these can be found in Figure A13 in Appendix A). The normal ranges were calculated assuming that 95% confidence limits were represented as the mean ± 1.96 standard deviation (Table 4.2).

*Table 4.2: Normal ranges for each of the platelet markers, derived from 20 healthy patients.*

| Platelet Marker | Normal Range (mean MFI ± 1.96 standard deviation) |
|---|---|
| CD62P | 197 ± 108 |
| C1q | 10.5 ± 5.5 |
| C3 | 10 ± 5 |
| IgG | 15 ± 10 |
| C3dg | 7.5 ± 8 |
| MPV* | 7.5 ± 1.5 |

*The units for MPV are fL.*

There is a documented MPV range quoted by Abbott as 5.6 – 12.1 (Gandhi 2019) and the MPV range calculated from the normal controls was 6 – 9 which is within this documented range.

### 4.3.2 Comparison of markers on pooled, apheresis platelets, normal controls, and haematology patients

Fifty-one samples each of apheresis and pooled platelet donations were tested for all markers to see if there were any significant differences between them. All platelets were irradiated, leucodepleted and transfused within 2 days of their expiry date. The results are shown in Tables A14 and A16 in Appendix A with the median and ranges calculated at the bottom of each table as the data was found to be non-parametrically distributed using the Kolmogorov-Smirnov test (Tables A15 and A17).

There were significant differences between the pooled, apheresis platelets, normal controls, and haematology patients for all the markers ($P<0.0001$) by the one-way ANOVA (due to the non-parametric distribution of the data).

The CD62P, C1q and C3 values in apheresis platelets were significantly higher than in both pooled platelets and normal controls and this can be seen quite clearly in Figure 4.2 which shows the median intensity fluorescence (MFI) comparisons of the target markers used. The haematology patients also had raised levels of CD62P like that of the apheresis platelet donations. Figure 4.2 (B) shows that the C1q levels in haematology patients are low and like that of pooled platelet donations, however, this could be a false assumption because the range illustrates that there were some very high values of this complement component on some platelets. There were higher IgG levels in the normal controls and haematology patients where EDTA was used as the anticoagulant prior to preparation of PRP. C3dg levels were raised in pooled platelet donations and haematology patients and slightly raised in apheresis platelet donations when compared to normal controls.

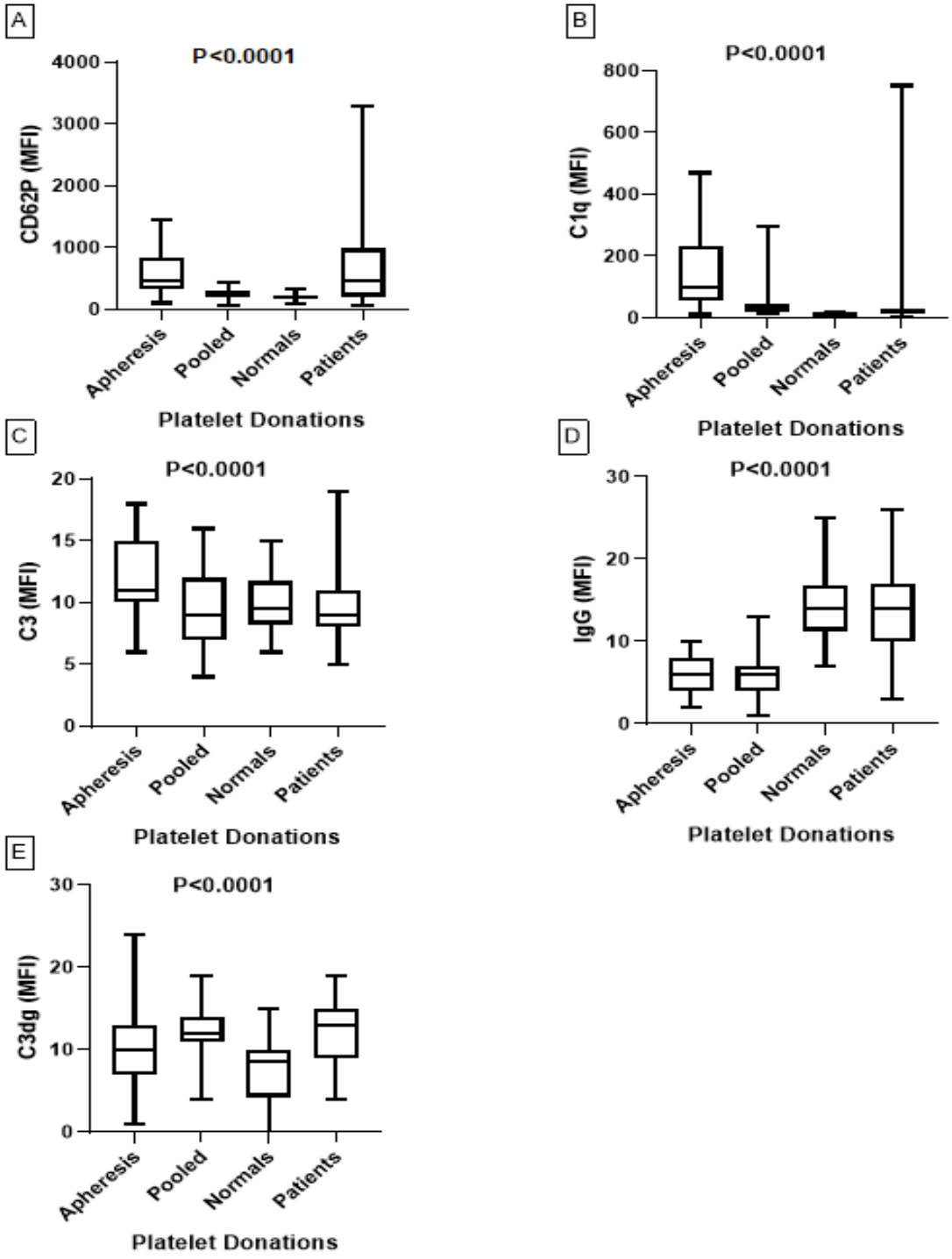


*Figure 4.2: This figure shows the difference in the MFI's of: (A) CD62P, (B) C1q, (C) C3, (D) IgG, and (E) C3dg expression on platelets from apheresis and pooled platelet donations, normal patients, and haematology patients. Statistical analysis was performed using the one-way ANOVA, given the non-parametric distribution of the data. Significant differences between the three groups are indicated. Apheresis platelet donations N = 51, pooled platelet donations N = 51, normal controls N = 20 and haematology patients = 103*

## 4.4 No correlation was found between MPV and CD62P levels in platelet donations

Due to the suggestion that an increased MPV could represent an increase in platelet activation (Gasparyan, Sandoo et al. 2010) (Korniluk, Koper-Lenkiewicz et al. 2019), graphs were plotted to see if there was any relationship between the MPV and the levels of CD62P in the two different types of platelet donation. The data was analysed for normality using the Kolmogorov-Smirnov test and found to be non-parametric, therefore, Spearman's rank correlation coefficient was used to see if there was correlation between the MPV and the levels of CD62P and the results from these can be found in Figures A18 and A19 in Appendix A. The range for the MPV in the apheresis platelet donations was 5 ± 0.9 which was slightly lower than for the pooled platelet donations (range = 6.2 ± 0.7). It has already been shown in section 4.2.2 that pooled platelet donations have a significantly lower level of platelet activation as represented by reduced levels of the platelet activation marker CD62P compared to that of apheresis platelets. The apheresis platelet donations show an inverse relationship where CD62P levels increase but the MPV decreases using Spearman's rank correlation coefficient showing r = -0.7 which is significantly different at $P<0.0001$. There was no obvious relationship between the increase in platelet activation and increased MPV, and in fact, these results indicate quite the opposite. Pooled platelets have a higher MPV (range = 6.2 ± 0.7) and a lower CD62P value, therefore, showing no relationship between platelet activation and the MPV value and no correlation with Spearman's rank correlation coefficient r = -0.05 (Figure 4.3).

If there was a clear relationship between platelet activation and MPV, a positive correlation between the two parameters would be observed.

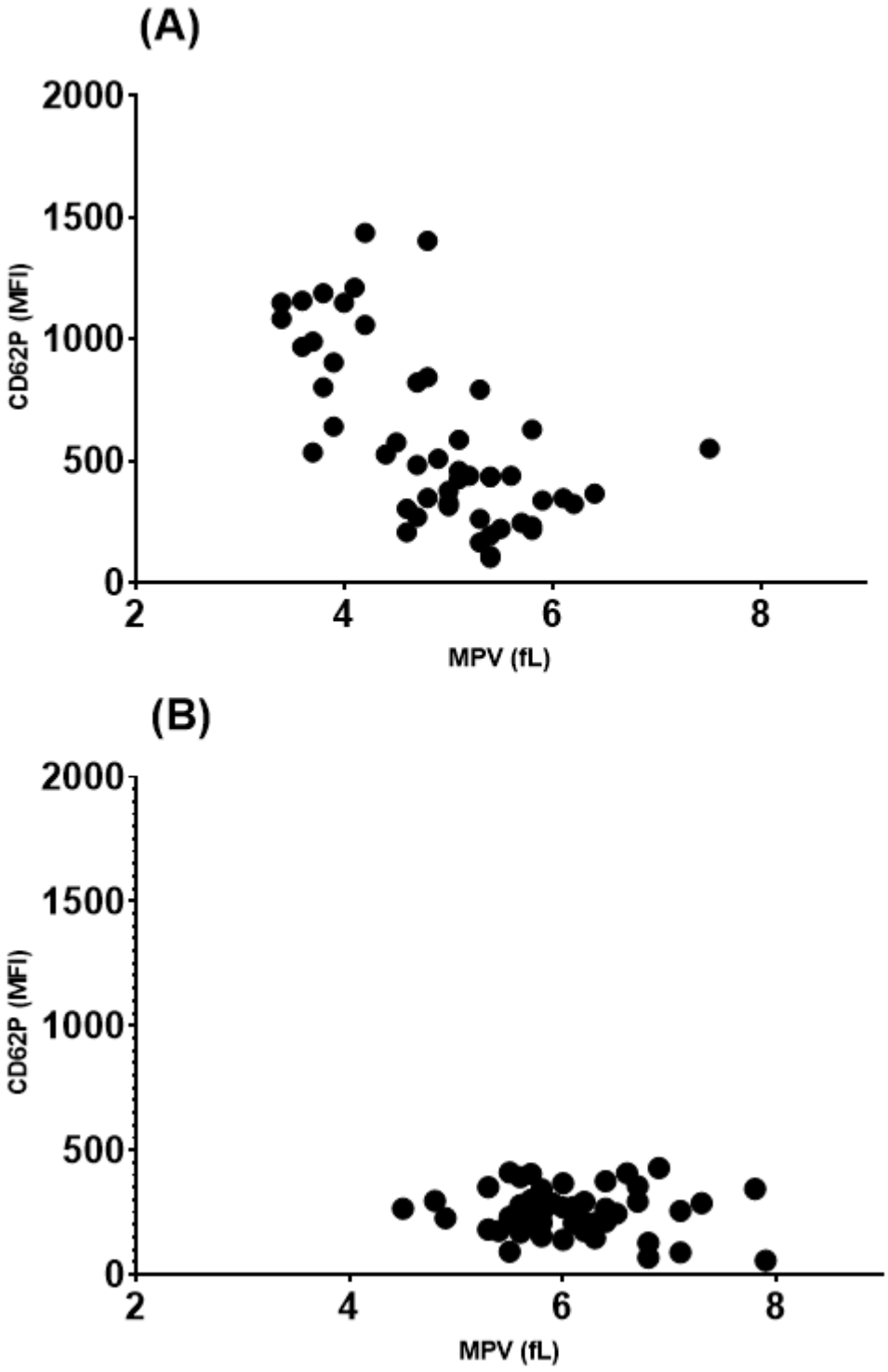


*Figure 4.3: This figure shows the relationship between the MPV and MFI's of CD62P using Spearman's rank correlation coefficient for (A) apheresis platelet donations (r = -0.7, significant at P < 0.0001, N = 51) and (B) pooled platelet donations (r = -0.05, not significant P > 0.05, N = 51). This figure clearly shows the higher CD62P levels in apheresis platelets compared to pooled platelet donations.*

## 4.5 Increased levels of CD62P and C1q in haematology patients and the possible effect on the platelet crossmatch

The MFI levels of IgG, C3b, C1q, C3dg and CD62P were put into tables for each patient and can be found in Tables A20-A27 in Appendix A.

For each patient it was important to show the effect of platelet transfusions on the values of CD62P and C1q as it was only these two parameters that were increased, and this can be seen in Figures 4.4 and 4.5. Patient 001 had very high levels of CD62P which remained above normal range during the transfusion episodes. Patient 002 also had high CD62P expression levels at the beginning of the transfusion episodes, which then moved into the normal range as transfusion occurred. Patient 004 and 006 fluctuated around the normal range with occasional increases. Patients 008 and 009 had the most transfusions during this study and both exhibited some very high CD62P levels. However, they also had some values within the normal range especially patient 009. Patients 010 and 011 had normal CD62P levels; however, those of 011 increased above the normal range after the first transfusion and remained high. In all these patients there did not seem to be an obvious pattern related to the transfusions as the levels varied quite a lot between them and over time. Both patients 008 and 009 had the most transfusions with at least one platelet unit being transfused every day for 6-8 weeks prior to the platelet count increasing adequately. The level of CD62P on their platelets did not specifically increase or decrease post-transfusion with a particular pattern. Approximately 50% of the transfusions led to a decrease in CD62P and 50% to an increase.

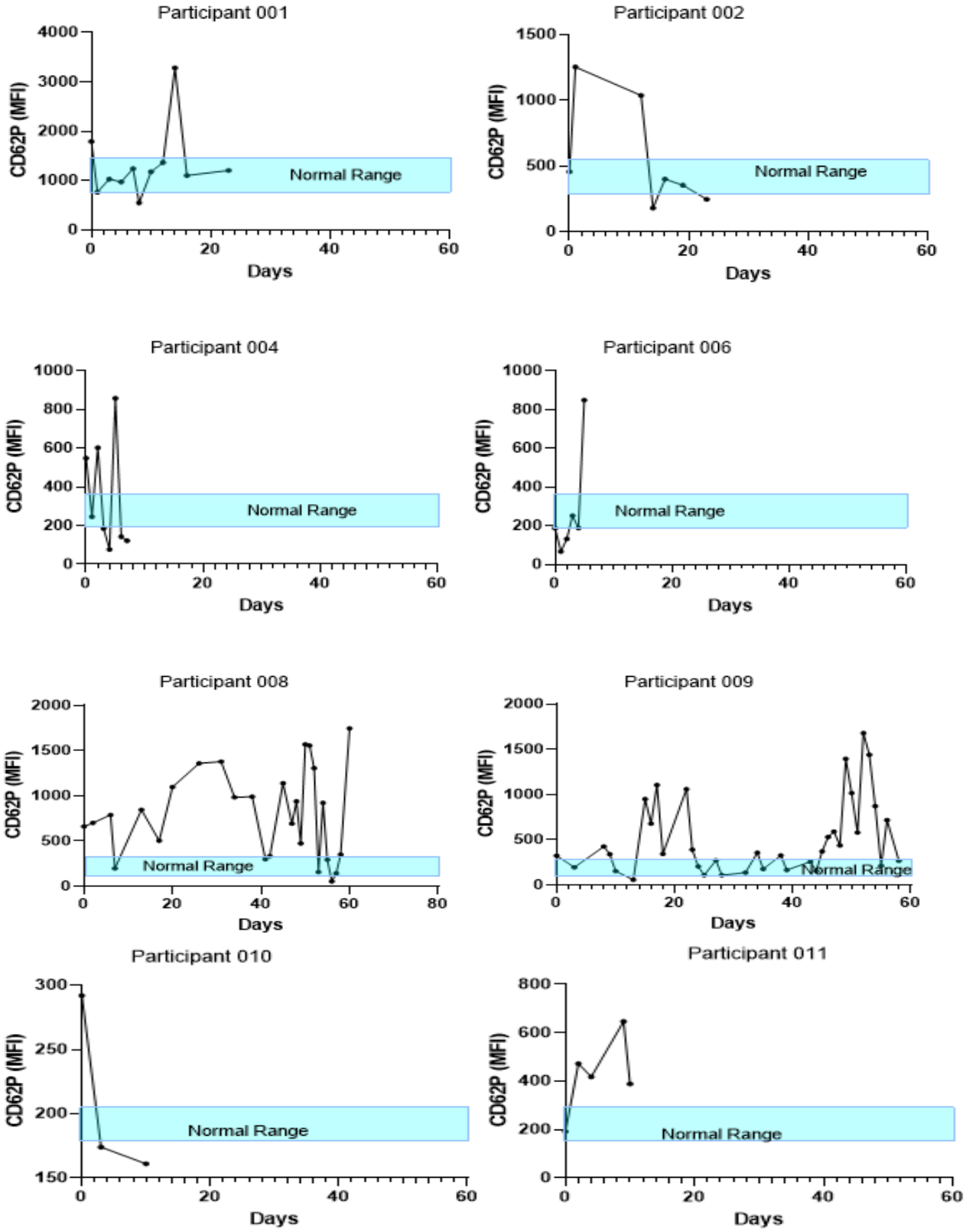


*Figure 4.4: These graphs illustrate the MFI (median fluorescence intensity) levels of CD62P in each of the patients as detected by flow cytometry and how they fluctuate post platelet transfusions. The in-house normal range has been added for comparison.*

Figure 4.5 shows the levels of C1q for all patients and how their results compared to the normal range. Patient 001 had the highest C1q values of all of them with some of the values reaching over 40 times higher than those in the normal range; however, these were high prior to transfusion. Patient 002 values remained within the normal range apart from the last one. Patients 004 and 006 had C1q values higher than the normal range but nowhere near as high as the first patient. Both patients 008 and 009 C1q levels fluctuated in and out of the normal range with some high peaks. In patient 010 the first platelet transfusion prompted the C1q value to increase massively but then returned to the normal range and patient 011 had values within the normal range. In all these patients there did not seem to be an obvious pattern related to the transfusions as the levels varied quite a lot between them.

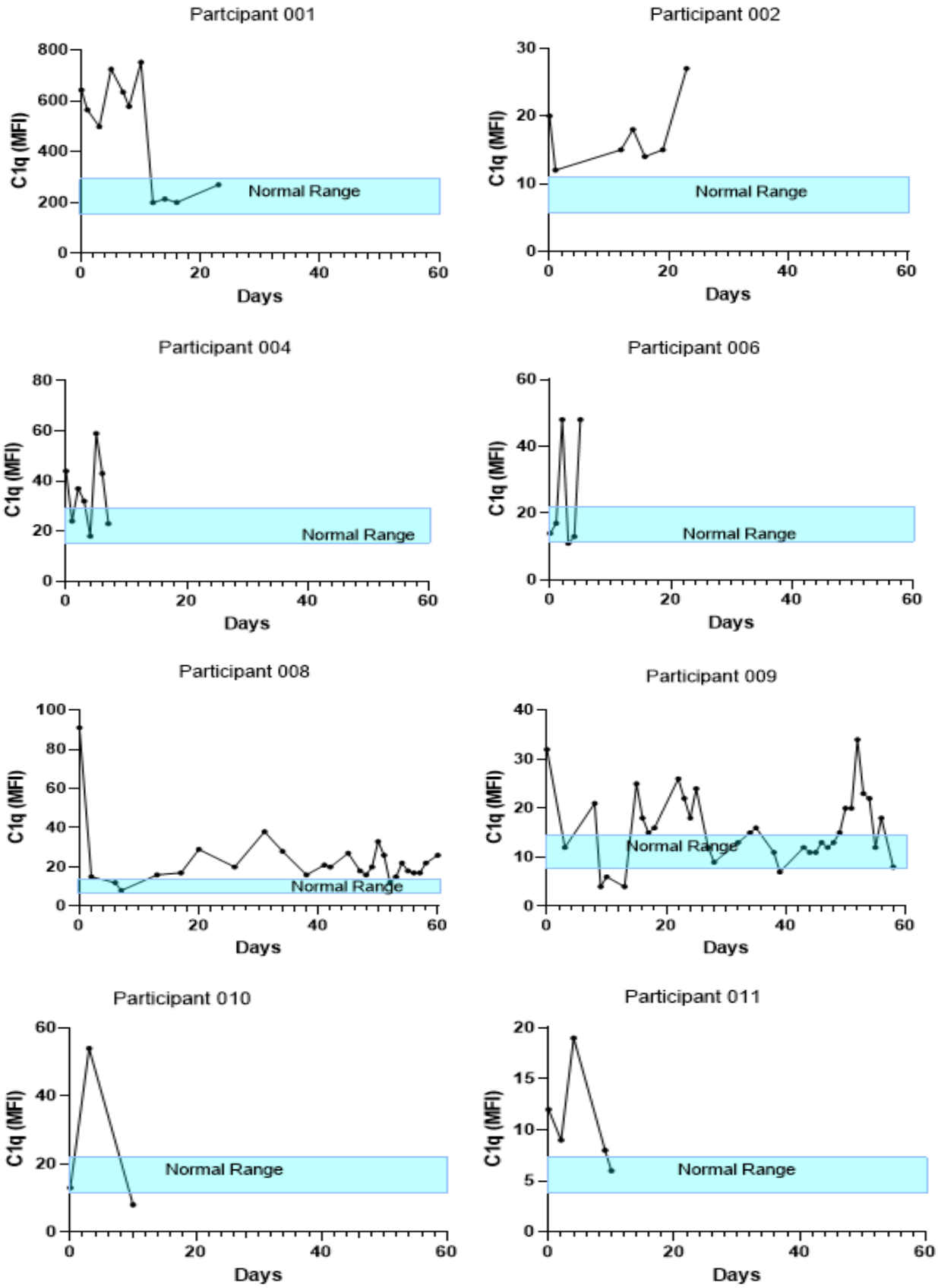


*Figure 4.5: These graphs illustrate the MFI (median intensity fluorescence) levels of C1q in each of the patients as detected by flow cytometry and how they fluctuate post platelet transfusions. The in-house normal range has been added for comparison.*

## 4.6 The increased levels of CD62P and C1q did not affect the crossmatch success

Out of the 96 platelet transfusions given to the haematology patients – 38 were apheresis and 58 were pooled platelet donations. Six of these were double transfusions and one triple transfusion was given, and these donations were not included in the crossmatch results or statistical analysis. From the original 96 donations, 15 of these were excluded since they were multiple transfusions on a single day leaving a total of 81 of which 47 were pooled and 34 apheresis donations. All information regarding the platelets transfused to patients, CD62P, C1q, MPV and crossmatch results are shown in Tables A28 and 29 in Appendix A. Out of the apheresis platelet donations, 53% (18/34) had C1q values above the normal range and 74% (25/34) had CD62P values above the normal range. For the pooled platelet donations, 30% (14/47) had C1q values above the normal range and 34% (16/47) had CD62P values above the normal range. The apheresis platelets showed higher levels of CD62P and C1q than the pooled platelets. There was no clear difference between compatible and incompatible results for CD62P in both types of platelet donation. However, in the case of C1q the incompatibility percentage in apheresis platelets was 3 times that of the compatible pooled platelets. This can be seen more clearly in figure 4.6 where the percentages are shown as a bar chart.

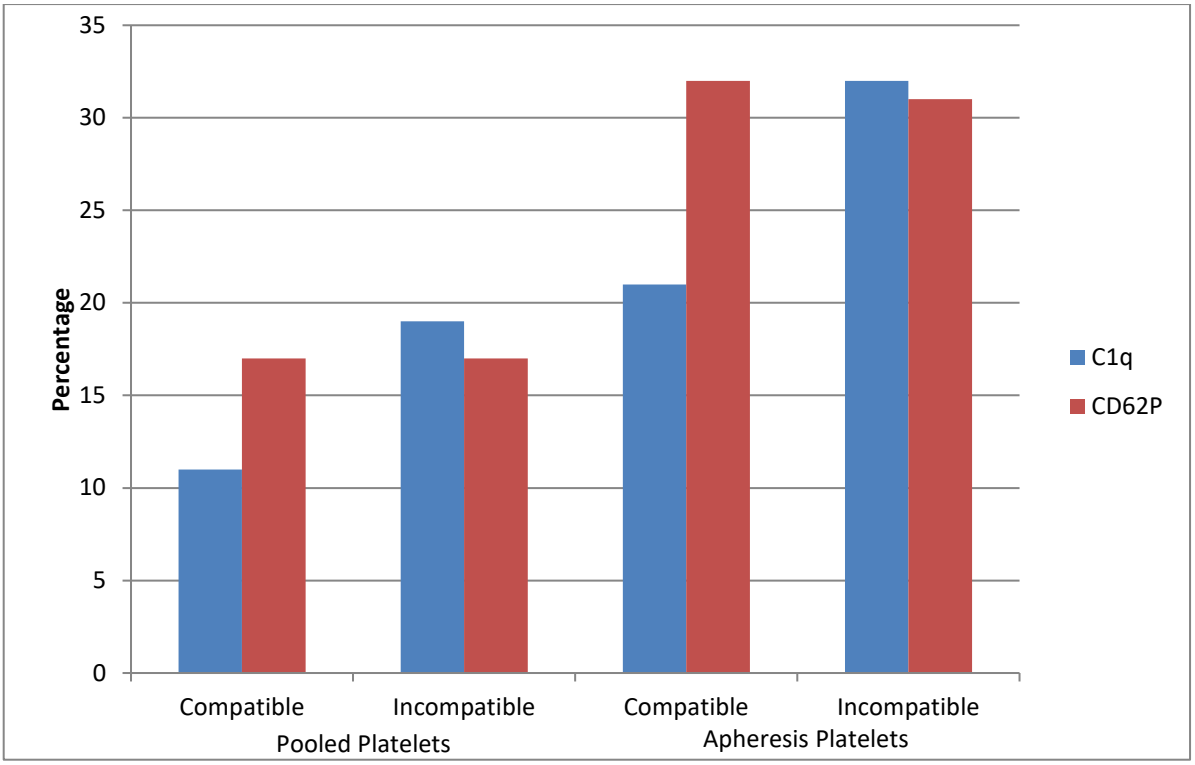


*Figure 4.6: Bar chart to illustrate the percentage of apheresis and pooled platelet donations that had increased levels of CD62P (red) and C1q (blue) above the in-house normal range and how those levels affected the crossmatch result.*

For the pooled platelet donations 30% (14/47) had C1q values above the normal range and 34% (16/47) had CD62P values above the normal range.

Previously the levels of CD62P in each of the patients has been shown (Figure 4.4), however, Figure 4.7 has the platelet donation CD62P levels added for comparison. Any of the values that are grossly abnormal have been labelled with C if that donation was compatible or an I if the donation was incompatible. Patient 004 and 010 both had one result higher than the normal range, but these donations were both compatible. Patients 006 and 011 had 2 donations with high CD62P levels which were both incompatible. Patient 009 had a few donations with values higher than the normal range, but they were a mixture of incompatible and compatible.

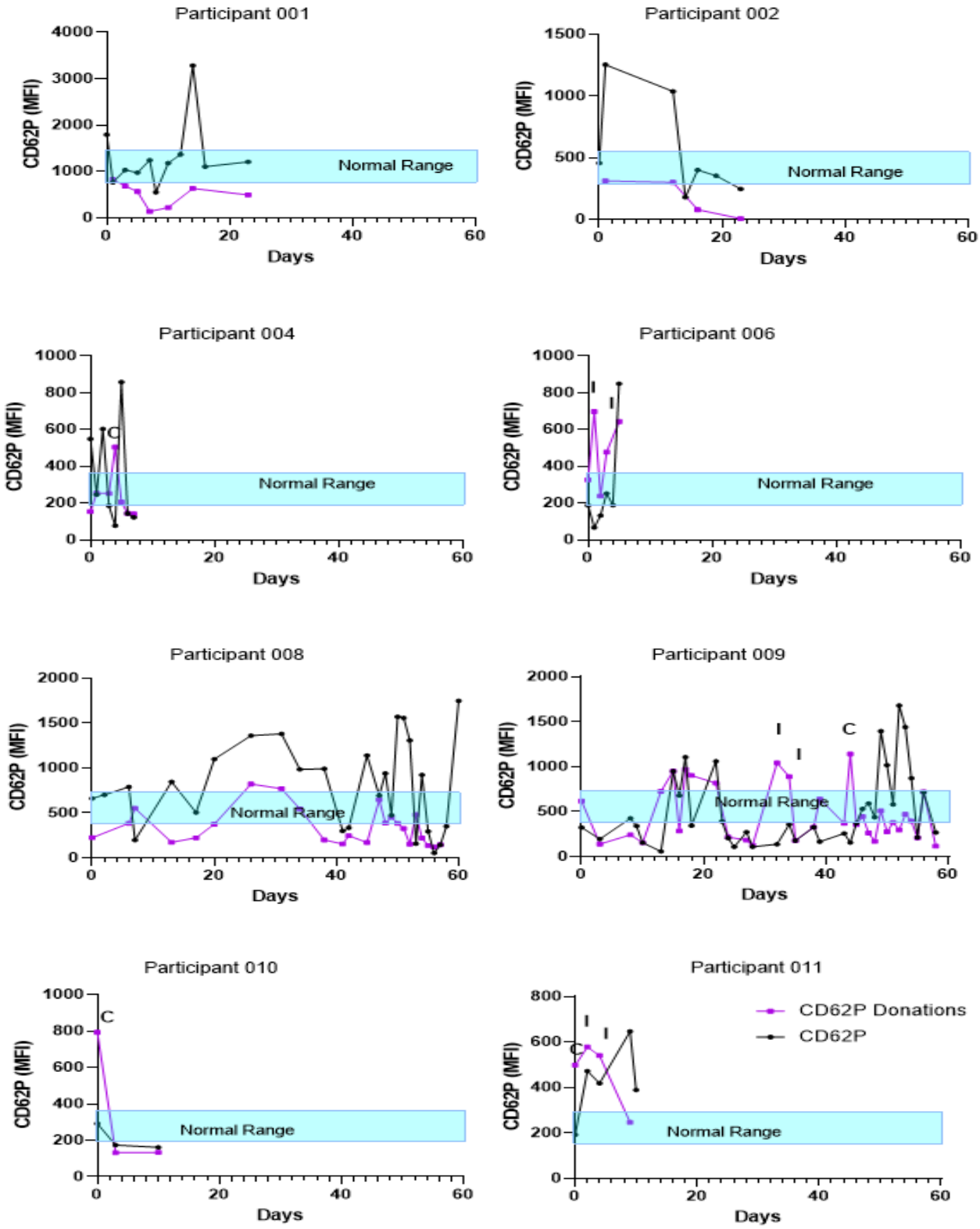


*Figure 4.7: These graphs illustrate the MFI (median fluorescence intensity) levels of CD62P as detected by flow cytometry and how they fluctuate post platelet transfusions in each of the patients (black line). The MFI levels of the platelet donations transfused (pink line) have been added to see if there was any correlation. The normal range has been added for comparison. The levels of CD62P in the platelet donations that are grossly abnormal are labelled with a C if compatible or I if incompatible with the patient.*

Previously the levels of C1q in each of the patients has been shown (Figure 4.5), however, Figure 4.8 has the platelet donation C1q levels added for comparison. Patients 001, 002, 004 and 006 all were transfused platelet donations with C1q levels within the normal range. Patients 008 and 009 donations had C1q levels that fluctuated around the normal range except a few high donations which were incompatible. Patient 010 had all the values within the normal range except the last one which was incompatible and patient 011 had donations that were all high and incompatible. None of the platelet donations with high levels of C1q were compatible with any of the patients.

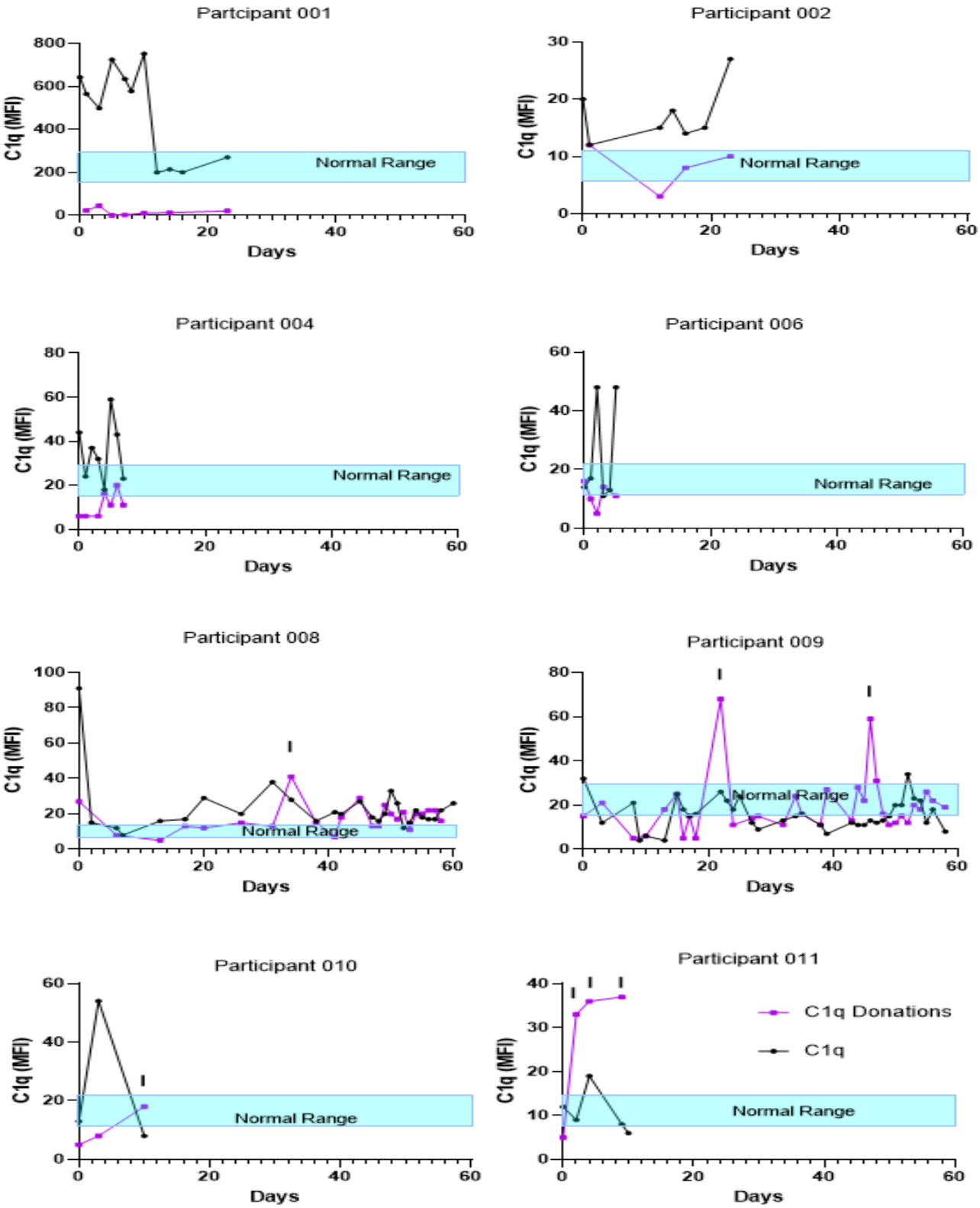


*Figure 4.8: These graphs illustrate the MFI (median fluorescence intensity) levels of C1q as detected by flow cytometry and how they fluctuate post platelet transfusions in each of the patients (black line). The MFI levels of the platelet donations (purple line) have been added to see if there was any correlation. The normal range has been added for comparison. The levels of C1q in the platelet donations that are grossly abnormal are labelled with a C if compatible or I if incompatible with the patient.*

The MFI values of CD62P and C1q were plotted on a scatterplot showing the median and range of the values as they were found to be non-parametric. Representing the data in this way shows that successful platelet transfusions did not have lower levels of CD62P or C1q, in fact they were slightly higher in this group (Figure 4.9). There was no correlation between CD62P and C1q results and a successful or unsuccessful crossmatch.

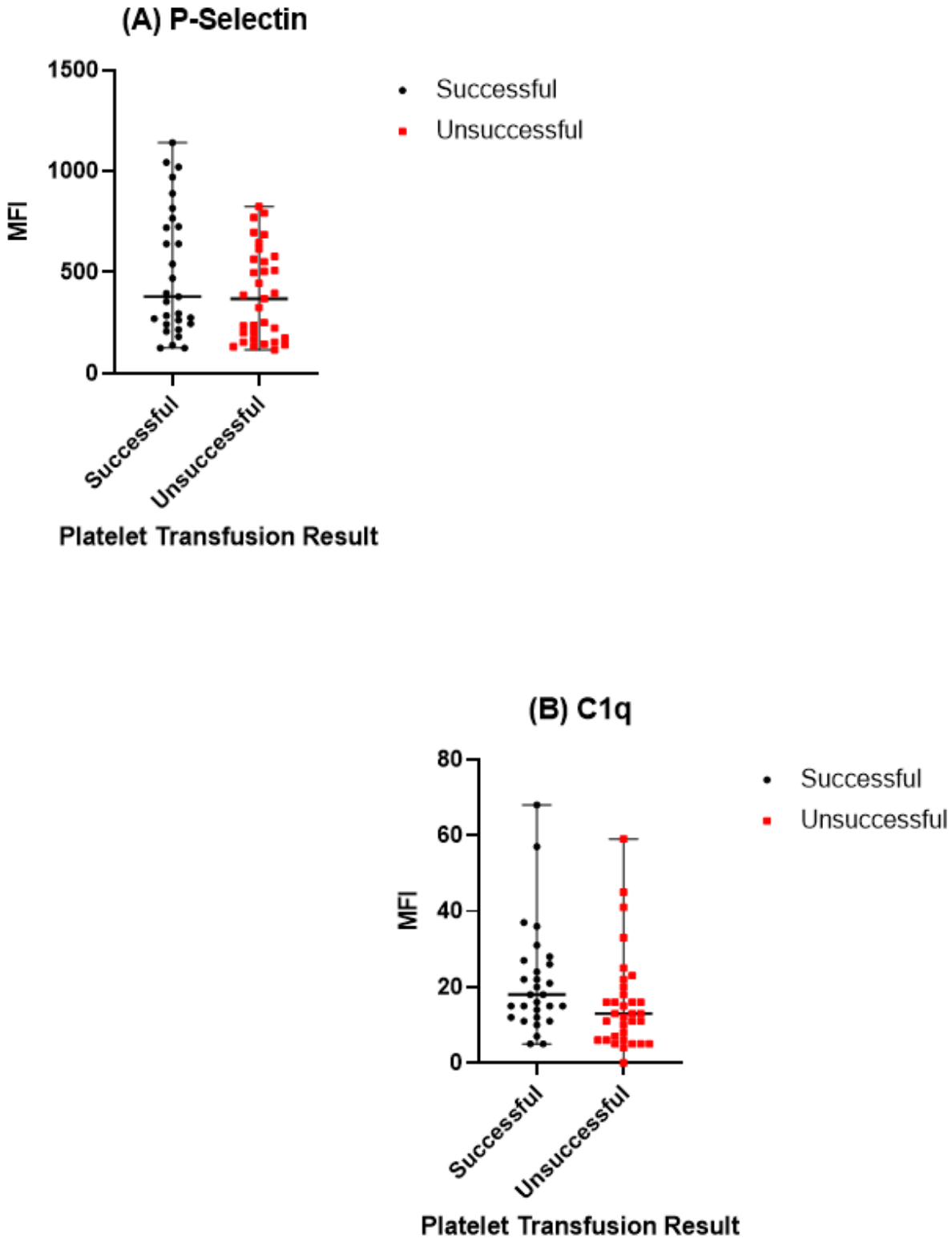


*Figure 4.9: Scatterplots showing the individual MFI (median fluorescence intensity) values for (A) P-Selectin and (B) C1q as detected by flow cytometry including the median and range and whether those transfusions were successful (black) or unsuccessful (red). This illustrates the wide spread of results and the fact that there was no correlation between increased values of CD62P or C1q and a successful or unsuccessful crossmatch*

## 4.7 Summary of Results

The results show that providing crossmatch compatible platelets did not improve platelet increments in the haematology patients of this study. The Capture-P crossmatch method used in this study was only 47% efficient, with both specificity and sensitivity being <50%. Markers of complement activation, P-selectin and MPV as markers of activated platelets and IgG were measured to see if any of these could account for the crossmatch results or transfusion outcomes. The CD62P, C1q and C3 values in apheresis platelets were significantly higher than in pooled platelets, normal controls, and haematology patients. Normal controls and haematology patients both had higher levels of IgG and the PRP for these were prepared from samples stored in EDTA anticoagulant which could account for this. Complement C3dg levels were higher in pooled platelets and haematology patients and slightly higher in apheresis platelets compared to normal controls. The MPV has been previously linked to an increased CD62P level due to the platelet shape change, however, in this study there was no correlation between the MPV and CD62P in pooled platelets and a negative correlation was found between the two markers in apheresis platelets. There were varying levels of compatibility between the platelet donations and the haematology patients with high CD62P and C1q values. There was no relationship seen with successful platelet transfusions and lower levels of CD62P or C1q, in fact they were slightly higher in this group.

# Chapter 5 Discussion

## 5.1 Introduction

The aim of this research was to see whether crossmatching and issuing compatible platelets improved platelet increments for haematology patients. Since patients with HLA/HPA antibodies were excluded from this study testing was extended to the measurement of markers of complement and platelet activation to see if this could be the reason for platelet destruction and account for any incompatibility encountered in the platelet crossmatch.

## 5.2 Summary of the study findings

Platelet crossmatching using the Capture-P method was a quick and easy assay with the manufacturer providing all reagents and the method being fully automated. In this study with 8 patients and 96 transfusion episodes the results illustrate that providing crossmatch compatible platelets does not improve the platelet increments. There was no evidence to suggest that crossmatched platelets would provide a better platelet increment than randomly issued platelets.

The immune reasons for incompatible crossmatches were thought to be eliminated by prior testing for platelet antibodies. However, complement markers (C1q, C3 and C3dg) and CD62P (platelet activation marker) could also account for the poor increments. The MPV was included as a possible marker of platelet activation as suggested by previous research (Gasparyan et al. 2011 and Korniluk et al. 2019); however, there was no correlation between the MPV and increased levels of CD62P. IgG antibodies were included with these measurements to ensure on-going monitoring of platelet antibody formation. Normal ranges were calculated using 20 volunteers due to a lack of published reference ranges for

these markers. When these were compared with those of apheresis and pooled platelet donations the CD62P and C1q results were significantly higher in the apheresis donations. Other differences seen were the slightly higher levels of C3 in apheresis platelets and C3dg levels in pooled platelets and the higher IgG levels in the normal controls where EDTA was used as the anticoagulant. However, the elevated levels of CD62P and/or C1q in platelet donations overall did not lead to incompatible crossmatch results – there was a mixture of compatible and incompatible results with normal and elevated levels of these markers meaning that these alone were not the reason for the incompatibility experienced. Platelet transfusions in haematology patients did not affect the levels of CD62P or C1q on their platelets, which were already higher than normal controls. Sometimes a platelet transfusion led to an increase or a decrease in these both or one of these markers – there was no specific pattern. Some haematology patients had elevated levels of CD62P with no obvious explanation and one haematology patient had an elevated level of C1q and this was maintained throughout the study with no obvious explanation.

## 5.3 General discussion of study findings

There are several factors responsible for the development of platelet refractoriness in patients including fever, sepsis, infection, DIC, bleeding, hypersplenism and HLA/HPA antibodies. Patients with immune refractoriness were thought to be excluded from this study, however, non-immune refractoriness still accounts for 60-80% of cases (Forest & Hod 2016) and it was possible that the haematology patients in this study could have had non-immune refractoriness at the time of testing. Since the platelets issued to all patients were within a day or two of expiry perhaps this could have caused a sub-optimal post transfusion platelet count in some patients. Also, the condition of the patient at the time of transfusion

and any underlying processes not accounted for could play a role.

The literature regarding the usefulness of platelet crossmatching was mixed with many different methods used, and conflicting results seen (Table A1 in the appendix).

Capture-P was used and found to be better for this study and for possible future use within the laboratory due to the high throughput and fully automated technology. It was easy to use with all reagents being supplied from the manufacturer and the only manual preparation required was to prepare the PRP and load the analyser. Calculations of the sensitivity and specificity from the data obtained in this study were 44% and 49% respectively with 47% efficiency. The specificity correctly identifies true negatives and sensitivity correctly identifies true positives. Therefore, this crossmatch method having a low sensitivity and specificity means that incompatible crossmatches should not be deemed as unsafe to transfuse and compatible crossmatches may not always produce good increments. This study revealed that the use of platelet crossmatching to provide compatible platelet products did not correlate with successful increments post-transfusion. This agreed with Wiita et al. (2012) who found no correlation between corrected count increment and percentage reactivity. However, it may prove useful as a bridging method until HLA-matched platelets became available and crossmatched platelets were used as a first line support for patients. Elhence et al. (2014) contradicted these findings and found that platelet crossmatching was superior, as any positive results they had were due to HLA or HPA antibodies and Salama et al. (2014) also decided that platelet crossmatching was the best predictor of transfusion response. Rioux-Massé et al. (2014) found that neither crossmatch compatible nor HLA-matched platelets provided better increments than randomly selected platelets. Crossmatched platelets have been used for emergencies with the unavailability of HLA-matched platelets (Chakrabarty and Das 2017). The drawback with

this approach is that many crossmatches may be required before a compatible one is found, and this would result in delays to treatment. Another important consideration is one of platelet stock availability, location, and the fact that it would not be practical for hospital sites to hold large stocks of platelets for crossmatching. Furthermore, there may be instances where no compatible platelets are identified for a patient and the stocks of platelets may then be wasted due to their short expiry dates. Once patients had produced HLA antibodies the crossmatch results were strongly positive in this study which then prompted platelet antibody testing. Since the immune reasons for platelet refractoriness had already been excluded it was thought not to be causing the incompatible crossmatches. Complement has been widely studied as being responsible for possible platelet activation in platelet donations but not in patients. Therefore, these markers were measured in both platelet donations and patients to see if they could be responsible for the incompatible crossmatch results.

A total of 8 haematology patients were used in this study and tested for complement markers C1q, C3 and C3dg, platelet activation marker CD62P and IgG. Since the use of platelet crossmatching in this study did not show a correlation between compatible crossmatches and successful increments the above markers were compared to see if they could offer any explanation for these results. All patients had levels of C3, within the normal range; however, most of them experienced raised levels of CD62P and C1q during this study. One patient in this study had very high levels of C1q which is suggestive of classical pathway activation. However, there was no relationship between the levels of CD62P and C1q and whether a platelet crossmatch was successful or not.

Both apheresis and pooled platelet donations were compared in this study to see if there were any significant differences between them which may then have consequences on the crossmatch result when transfused to haematology patients. The apheresis platelet donations had CD62P, C1q and C3 values that were significantly higher than in the pooled platelet donations ($P<0.0001$) and the range of these values was wide. There are different machines used for collecting apheresis platelet donations with similar technology which involves centrifugation and filtration principles where platelets come into close contact with membranes that can potentially cause platelet activation (Wun et al. 1992). Increased levels of platelet microparticles have been seen in apheresis platelets and these are determined by the methods of collection, pathogen reduction technologies and apheresis technology used which may expose platelets to different levels of physical stress (Millar et al 2020). This study by Millar et al (2020) was limited to looking at two different cell separator technologies for collecting apheresis platelets only and there was no comparison with pooled platelets.

It was concluded in this study after the comparison of platelet donations that the apheresis platelets had higher levels of CD62P on the platelets and therefore increased levels of activated platelets. However, out of the total of 34 apheresis transfusions 61% were compatible and 39% were incompatible. The results of the platelets being transfused to the haematology patients show that the apheresis platelets do have a much higher level of CD62P than pooled donations, but this does not seem to produce incompatible results as there are those with very high levels that are still compatible. According to the literature (Labrie 2018) increased platelet activation should produce lower increments due to their

removal from the circulation by macrophages. Complement components such as C1q and C3 are opsonins enabling macrophages to recognise them for phagocytosis.

Apheresis platelets contain 100% plasma which will contain complement components that can also play a part in platelet activation and destruction. This could account for the increased levels of C1q and C3 seen in apheresis platelets. The increased levels of CD62P in apheresis platelets were indicative of increased platelet activation in these donations. Whereas pooled platelets being prepared from buffy coats and plasma from different donations experience some degree of activation but then most of the plasma is replaced with PAS leaving only approximately 35% plasma which will remove some of the complement components and other biological mediators. De Wit et al., (2022) found that platelet concentrates in PAS's generated less complement activation products during storage than platelets stored in plasma. Even though activated platelets were found in some platelet donations and haematology patients as evidenced by increased CD62P expression in this study – there was minimal binding of C3 which conflicted with the findings by Del Conde et al (2005). Perhaps the C3 did not bind, was destroyed, or did bind and subsequently came off prior to measurement.

Del Conde et al. (2005) noted an increase in C3 upon platelet activation and confirmed that C3b binds to the surface of activated platelets. It is likely that most of this C3 was stored in α-granules, and that the increase in C3 upon platelet activation represented deposition of the protein on the platelet surface. They also identified P-selectin as a C3b-binding protein that is sufficient for activating the complement system. Gyongyossy-Issa et al. (1994) found that the percentage of C3 positive platelets peaked at the third day of storage but had declined by day 5. All the platelets tested in this study were within a day or 2 of their

expiries and maybe the C3 that could have originally been present on the platelets had detached or been destroyed in the pooled platelets. In addition to this, platelets express specific binding sites for C1q which is part of the first component of the classical complement pathway (Peerschke and Ghebrehiwet et al. 1987, Ghebrehiwet et al. 1996). Skoglund et al. (2009) suggested that a moderate increase in P-selectin expression upon C1q stimulation observed in their study was sufficient to trigger further activation of complement, without leading to massive platelet activation. The question is whether complement deposition on platelets could be a novel biomarker for a thrombotic process and requires more understanding in terms of the underlying mechanisms involved.

C3b is degraded to C3dg, and this is a poor opsonin – like a positive direct antiglobulin test (DAT) result on red cells where C3d is present. It shows that some complement activation has occurred. There was a slight increase in the levels of C3dg found on platelets in pooled platelet donations and in haematology patients compared to apheresis platelets.

Perhaps the preparation methods involved in the platelet production processes or PRP resulted in some level of complement activation. Miletic and Popovic (1993) found that the amount of C3dg fragments on platelets gradually rose during the first 3 days of storage. At the end of 5 days of storage, the platelets became C3dg negative. There are two possible mechanisms of C3dg disappearance--shedding and/or further degradation of C3dg fragments. Those results indicated that complement activation and the generation of complement-dependent ligand-receptor interaction may be mechanisms for platelet activation in concentrates stored at room temperature. Chen et al. (1994) found that C3dg binds to human platelets in a specific and saturable manner. The direct interaction of platelets with soluble C3dg may contribute to immune-mediated platelet destruction. More

importantly, platelets may interact with opsonized pathogens or complement-activating immune complexes via C3dg.

Recent literature by Chu et al (2021) found that pooled platelet concentrates provided a small benefit over apheresis platelets. As a rule, most hospitals tend to switch to apheresis platelets as they are single donors, and this was hoped to minimise alloimmunisation. However, in the study above the clinicians switch to pooled platelets based on the hypothesis that greater HLA diversity may partially spare transfused platelets from immune-mediated clearance. In support of this they observed a small but potentially clinically relevant benefit in transfusing pooled platelets as evidenced by improved increments and interestingly this study only included 7 patients. However, Friedberg et al., (1996) found that data from outcome studies presented indicated that increased reliance upon single donor apheresis platelets at the expense of pooled random donor units can improve the overall quality of transfusion practice by decreasing platelet utilisation, resource consumption, donor exposures, and platelet wastage.

In terms of preferentially using pooled platelets there will be other potential disadvantages over apheresis platelets including increased donor exposure to infectious disease transmission risk and increased donor exposure to HLA (Chu et al 2021).

The normal controls and the haematology patients showed higher IgG levels, and this could be because these platelets were collected into EDTA instead of ACD or PAS. Hedge et al (1984) measured platelet associated IgG in samples stored in four different anticoagulants and found that those in EDTA gave slightly higher initial values (day 0) than in other anticoagulants. Lucas et al (1982) found that normal levels of platelet associated IgG depended upon the anticoagulant used for blood collection and were on average five-fold

higher for blood taken into EDTA than for blood taken into ACD. Samples collected into EDTA alone were found to be least satisfactory, with a rise in platelet associated IgG by 24 hours after collection (Gibbons et al., 1982). Since samples stored in EDTA were used for the preparation of PRP in normal controls and haematology patients it is possible that this could account for the higher levels of IgG measured in this study and further comparisons would be needed to prove this. The increased IgG levels on the normal control samples maybe due to the small number used to obtain a normal range for IgG which allows for greater variability. Also, the normal controls were presumed to be normal volunteers but may have had unknown higher IgG levels.

Due to the shape changes that take place, the MPV has been suggested as being a measure of platelet activation (Gasparyan et al. 2011 and Korniluk et al. 2019). In this study the MPV's were compared with the levels of CD62P as the marker of platelet activation to see if any relationship existed within the platelet donations sampled. The range for the MPV in the apheresis platelet donations was 5 ± 0.9 which was slightly lower than for the pooled platelet donations (range = 6.2 ± 0.7). Using Spearman's rank correlation coefficient r = -0.7 which is significantly different ($P<0.0001$) for the apheresis platelets so the higher the MPV the lower were the levels of CD62P. The pooled platelets gave a correlation coefficient of r = -0.05 ($P>0.05$) showing no correlation at all. Therefore, there was no correlation between the increase in platelet activation and increased MPV for donated platelets in this study. Upon exposure of platelets to various biological stimuli and agonists, shape change occurs via dismantling and reorganization of the cytoskeletons. First, platelets become spherical as actin filaments are fragmented, which is followed by the dismantling and release of spectrin networks composed of filamin A, GP1b/IX and spectrin. Second, the membranes of the cells

protrude due to actin filament assembly, which manifests as pseudopodia (Sandmann and Koster, 2016). When platelets were sampled, they could be at any of the above stages and therefore the MPV could be increased in some as they start to swell. Holme et al. (1997) found that stored platelet concentrates in which no loss of viability had been demonstrated showed pronounced P-selectin expression associated with platelet swelling and loss of discoid shape. They were also able to show that after incubation at 37$^{o}$C the P-selectin levels returned to control levels with a reduction in swelling and normal discoid shape.

## 5.4 Study limitations

The timing of the pre and post platelet counts could be improved in future work with more rigorous selection of the patients. The incorporation of the broad selective term 'haematology' patients, the number tested, and the timing of sample collection were weaknesses of this study. Haematology patients were used in this study; however, this is a broad term which incorporates many haematological conditions. Perhaps this should have been narrowed down to post allogeneic transplant patients who often develop platelet refractoriness. Limitations of this research included the small sample number (8) possible mixed immune and non-immune etiologies of refractoriness among patients and varying blood sampling times.

Most of the research involving platelet preparation methods reports the collection of whole blood into sodium citrate tubes. In this study peripheral blood from consented adults was drawn into tubes containing EDTA (Ethylenediaminetetraacetic acid) as an anticoagulant as it is reported that this prevents further complement activation (Sinosich et al. 1982 and Pfeifer et al. 1999). This study involved the measurement of complement markers on platelets, so blood collected into EDTA was used and this was also the sample required for

the platelet count and therefore limited the number of samples being collected from the patients. However, this could have caused the increased levels of IgG seen in the normal controls and haematology patients which may not have been an issue if PRP had been prepared from samples using sodium citrate instead of EDTA for the anticoagulant.

Generally, to establish a new reference range a minimum of 120 samples is required (Wayne 2010). For verification of an established range, a minimum of 20 samples is required. In this study only 20 samples were used to establish the normal ranges of all markers due to availability at the time due to a current pandemic. This is obviously a limitation as there are no reference ranges quoted in the literature for these markers and 20 samples is insufficient and increases the variability.

Many studies have reported using various buffers for final resuspension of platelets, including both HEPES (4- (2-hydroxyethyl)-1-piperazineethanesulfonic acid) and Tyrodes buffer. HEPES buffer was tried in this study, however, it was found that the platelets were difficult to resuspend. They tended to aggregate at the bottom of the tube causing issues with downstream tests. This study therefore moved to using the Immucor platelet wash and PWSS which is a buffered phosphate solution containing sodium chloride and stabilisers to minimise platelet aggregation. The PWSS did not cause clumping problems and the platelets gently went into solution when mixed on a platelet agitating machine. Once platelets had been washed and suspended with PWSS they were used immediately or stored at 1-10$^{o}$C for up to eight months prior to use, however, all specimens were brought to 18-30$^{o}$C before processing. The platelets once suspended in PWSS were tested immediately and no problems were encountered when using this solution.

Most studies mentioned fixing the platelets prior to testing to arrest any further activation that may occur during the processing steps, however, Wun et al. 1992 mentioned that P-selectin antibodies did not bind as well after this as they found there was a decrease in the binding of monoclonal antibodies. Cahill et al. (1993) described a series of experiments comparing platelet activation antigen expression detected by flow cytometry in fixed and unfixed samples. Formaldehyde increased the expression of P-selectin and was not recommended when studying platelet activation. The decision was made not to fix platelets to prevent further activation, however, the brake was not used when centrifuging platelets and care was taken when washing platelets such that they were gently resuspended using a platelet agitator.

## 5.5 Suggestions for Future Work

When comparing platelet donations further information on the donors themselves, their medical history and drug history would be useful as a future research study. Platelet activation status is primarily related to donor characteristics, and this can be affected by factors such as exercise, diet, and other pathological conditions. One study looking at the *in vivo* recovery of transfused autologous platelets (Stefely et al. 2020) found that multiple measurements of transfused platelet recovery were relatively consistent within individuals but differed between them. In fact, the clinical platelet transfusion corrected count increments varied depending on the donor and the storage time.

Including measurement of platelet microparticles could ensure that these did not interfere with the platelet measurements alone. These are approximately 0.5µm in size and express glycoprotein IIb/IIIa and tend to be found in platelet poor plasma. Only platelet rich plasma was used in this study to isolate platelets, however, there is a chance that some platelet

microparticles may have been present, but hopefully these would have been removed using the gating strategy explained in the methods. Rank et al. (2011) investigated the presence of platelet microparticles in apheresis and pooled platelets and found that they both contained these and their levels increased over storage time.

Measuring levels of other markers such as soluble P-selectin could have been incorporated into this study as once the P-selectin is expressed on platelets it is then released into the supernatant and comparison of both values together may be useful. Dunlop et al. (1992) demonstrated that soluble P-selectin circulated in normal plasma in a soluble and potentially functional form and is secreted from activated platelets.

Instead of just looking for activated platelets and whether they have complement present maybe the platelet viability should be taken into consideration. However, Bakry et al. (2010) stated that P-selectin expression on platelet surfaces correlates with loss of viability upon transfusion of the stored platelet concentrates. Bikker at al. (2016) also looked at functional recovery of stored platelets after transfusion and mentioned that most P-selectin positive platelets were most likely cleared from the circulation upon transfusion. More studies on platelet function before and after transfusion would be more useful. Michelson et al. (1996) demonstrated that activation markers are lost from the surface of transfused platelets as they circulate, and that platelet activation does not apparently induce loss of *in vivo* viability. Therefore, there is a need for more clinical studies to clarify whether the observed *in vitro* differences we are all finding between pooled and apheresis platelets have clinical impact or are just *in vitro* observations.

It would have been useful to test platelet donations every day up until their expiry date – however, platelet use within this hospital is very high and the minute platelets are ordered

and available they are used immediately. If they were tested daily more information could be gathered on the levels of all markers as the platelets age.

Stored platelets will contain other biological mediators – maybe the stored supernatant should be removed from the platelets to improve clinical outcomes like the production of pooled platelets where the plasma is replaced with PASsolution. This could prevent unwanted substances such as soluble P-selectin, platelet microparticles and CD40L accumulating during platelet storage and subsequent events happening because of transfusing these (Stolla et al. 2015).

A cost analysis of crossmatching platelets and issuing compatible donations rather than issuing random donations could be undertaken. Only one study from the literature search evaluated platelet transfusion utilization, resource use and costs in a tertiary care hospital over a 6-month period (Meehan et al 2000). Clinical and financial data were collected, evaluated, and compared to identify trends in resource utilization based on admitting service and platelet-refractory status. This highlighted the significantly greater costs associated with patient's refractory to platelet transfusions in terms of product support and lengths of stay. Freedman et al. (1989) found that HLA-matched platelets were relatively cost-inefficient in comparison to the crossmatch compatible platelets.

## 5.6 Summary

In summary, pooled platelet donations may become a more favorable choice for haematology patients due to the increased levels in complement markers and CD62P seen in apheresis platelet donations. Even though systematic reviews of the clinical studies evaluating different techniques for selecting HLA compatible platelets have not been

powered to demonstrate improved clinical outcomes, platelet refractoriness is currently managed by the provision of HLA-matched or crossmatched platelets (Stanworth et al., 2015).

Platelet crossmatching represents a technology change involving clinical practice and suggestions for incorporating this test into platelet transfusion algorithms should be considered (Menitove, et al., 1995). It would be useful to build an algorithm within the hospital for the treatment of patients with suspected platelet refractoriness. Three papers (Kopko et al. 2015; Juskewitch et al 2017: Cheok et al 2020) gave examples of algorithms put together for the management of their patients. All three of these incorporate immune and non-immune factors, platelet increments, HLA-matching, and platelet crossmatching. Maybe HLA-matching and crossmatching could work together in the patients favour and reduce alloimmunisation so that crossmatched platelets could be used as a bridging way of providing platelets until HLA-matched platelets were available. Clinical outcomes of the patients were beyond the scope of this study; however, further research could show possible relationships between *in vitro* results and clinical outcomes. Patient-related factors are the main determinants of the effectivity of platelet transfusions. Platelet manufacturing and storage methodologies are constantly evolving and trials using refrigerated and cryopreserved platelets which could extend their shelf-life to weeks, or more are now being investigated. Perhaps new assays focusing on platelet viability in the platelet donations are required in addition to other tests carried out. More clinical studies are needed to evaluate the haemostatic potential of stored platelets and the amount of pro-inflammatory and pro-thrombotic mediators in the supernatant. Haemostasis was always considered to be the primary role of platelets; however, research has now highlighted that their function is more

complex involving roles in regulation of the immune system. This research could help drive the next generation of treatments and care for haematology patients by influencing the development of a policy, practice, and service provision.

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# Appendix

## Appendix A

*Table A1: A representation of the outcomes of 18 studies looking into providing crossmatch compatible platelets for refractory patients.*

| Author and Year | Sample Numbers | Sample Source | Methods | Platelet Products | Evaluation of Crossmatches | Assay Performance | Cost per Crossmatch | Findings/Problems |
|---|---|---|---|---|---|---|---|---|
| Brand et al., 1978 | 15<br><br>10 Male<br>5 Female | Bone marrow failure.<br>Non-immunes excluded | PIFT<br>LCT | HLA-matched | Total = 82<br><br>72% = compatible<br>35% = not compatible<br><br>After 4 units found not compatible patients were considered allo-immune.<br>7% were non compatible by PIFT | Sensitivity = 79%<br>Specificity = 100%<br>NPV = 90%<br>PPV = 100% | NS | PIFT failed to pick up weak HLA antibodies.<br>HPA antibodies not mentioned/tested for – and therefore could account for the false positives.<br>HLA matching can fail to give an adequate transfusion response. |
| Kickler at al., 1983 | 19<br><br>8 Male<br>11 Female | Aplastic anaemia, AML, bone marrow transplants. | PRAT<br>LCT<br>Assay QC | HLA matched<br><br>4 were not ABO | Total = 89<br><br>26 transfusion failures | Sensitivity = 27-96%<br>Specificity = 77-100%<br>NPV = 77-98% | NS | Transfusion failures seen even though the platelets were HLA matched.<br><br>Found LCT unsatisfactory with |

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| | | Non-immunes excluded | | matched | 63 = satisfactory | PPV = 27-100%<br>Accuracy = 65-89% | | a 35% crossmatch error rate. |
| Kickler et al., 1985 | 42<br><br>Did not specify whether males or females | Haematology patients.<br><br>Non-immunes excluded | PRAT | HLA matched | Total = 230<br><br>54-60% negatives were transfused | Sensitivity = 82-86%<br>Specificity = 84-92%<br>NPV = 83-92%<br>PPV = 73-91%<br>Accuracy = 84-87% | NS | Despite using HLA-matched platelets unsuccessful transfusions still occurred.<br>Selections of serologically cross-reactive antigens similar to the recipient can result in failure.<br>It was mentioned that HLA matching of large pools of patients is expensive. |
| Freedman et al., 1989 | 100<br><br>Did not specify whether males or females | AML patients.<br><br>All had platelet antibodies.<br><br>No mention of non-immune factors | LCT<br>ELISA<br>PIFT<br>PRAT<br>LCT+PRAT | Pooled | Total for each<br>48<br>48<br>56<br>61<br>48<br><br>Each crossmatch represents 12 units per patient | Sens Spec Acc<br>62 79 71<br>28 84 44<br>40 81 55<br>79 67 71<br>93 47 NS<br><br>NPV - NS<br>PPV - NS | Included costs per crossmatch in dollars.<br><br>Certain assumption made for the costing analysis | This study included costings as which are imperative for detailed analysis of the treatment of haematology patients.<br>The assumptions made in this study need to be validated by further research.<br>HLA-matched single-donor platelets were relatively cost-inefficient in comparison to the crossmatch-compatible platelets. |
| O'Connell et al., 1990 | 41 | AML patients | Capture-P used for 8 | Pooled | Total = 964<br><br>24% negatives | NPV = 30% | NS | A very small number of patients were crossmatched by capture-P and more by |

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| | | | patients<br><br>ELISA used for 33 patients | | were transfused | | | ELISA. Even though similar results were obtained by both methods it is unclear why they were used in this way. |
| Moroff et al., 1992 | 73<br><br>46 male<br>27 female | Haem/Onc<br><br>Non-immunes excluded | Solid phase RIA PIFT | HLA matched Vs cross-matched Apheresis | At least 10 crossmatches for each patient. Any needing more than 40 units of platelets crossmatched were excluded from the study. | None | NS | Difficulties of supplying HLA matches sometimes due to polymorphism of the HLA system. HLA grade is a factor in the success of the transfusion. HLA matched is best but difficult to maintain. False negative crossmatches were due to antibodies not detected. |
| Gelb et al., 1997 | 66<br><br>33 male<br>33 female<br><br>16 died<br><br>Mixture of adults and paeds. | Haem/Onc and platelet disorders, carcinomas.<br><br>No antibody testing done in this study.<br><br>Immune and non-immune factors included. | Solid phase | | 475 crossmatch compatible units went to the 66 patients.<br><br>10 patients had no negative crossmatches due to antibody production. | None | NS | Crossmatch compatible platelets significantly improve the mean CCI for 50% who are refractory to random donor platelets even when they are not pre-selected for alloimmune refractoriness. |
| Rebulla et | 480 | Haem/Onc | Capture- | Buffy | Total = 569 | NPV = 68% | Cost per | Noted that women were more |

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| al., 2004 | 228 male<br><br>252 female<br><br>18 deaths | adults<br><br>40 Already had HLA+/- HPA | P<br><br>Took 160 minutes to crossmatch one patient against 94 platelet suspensions. | coats | No negative donors for 12%. | | crossmatch kit was mentioned. | refractory.<br>Platelet count increments were significantly higher than those in the same patients given 303 transfusions from random donor platelet pools. |
| Sayed et al., 2010 | 39<br><br>Adults and Paeds | AML<br><br>Non-immune factors not mentioned | PIFT<br><br>QC | ABO matched Apheresis<br><br>HLA matches were done for 30 transfusion events | Total = 60<br><br>52/60 were transfused<br><br>31 males and 29 females. | | NS | Crossmatch compatible platelets associated with a good response in 51% of transfusion events. Non-crossmatch platelets gave a poor response in 83% of events. CCI's were studied at 1, 18 and 24 hours post transfusion. |
| Wiita et al., 2012 | 71 | Haem/Onc patients.<br><br>Non-immunes not excluded. | Capture-P | Apheresis | Total = 443<br><br>41% compatible gave increased CCI's<br><br>4 crossmatch | NPV = 41%<br><br>No correlation between CCI and % reactivity. | NS | Crossmatch compatible was the first line of support.<br>738 crossmatch compatible units given to 71 patients.<br><br>This study showed no increased alloimmunisation |

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| | | | | | assays per patient.<br><br>% reactivity in the wells was used to ID any that had possible HLA antibodies. | | | because of providing crossmatched platelets and was a good alternative to the gold standard of matching for HLA antigens. |
| Elhence et al., 2014 | 31<br><br>20 male<br>11 female | Aplastic anaemia and AML | Capture ELISA | | Total = 100<br><br>56 incompatible<br>44 compatible | | | Crossmatch compatible platelets are associated with adequate CCI in 92% of transfused units. Crossmatch incompatible platelets are associated with a poor response in 39% of transfusion events. |
| Jia et al., 2014 | 193<br>98 female<br>95 male | Haem/Onc and other diseases | Solid Phase<br><br>Antibodies identified with solid phase and then crossmatched. | Apheresis<br><br>ABO comp | Total = 88 | | NS | There were difficulties getting compatible platelets for patients – only crossmatched 4 units for each patient due to staffing issues and limited time.<br>Significant CCI's were found after crossmatch compatible units were transfused. |

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| Salama et al., 2014 | 40<br>16 male<br>24 female | Haem/Onc patients with immune and non-immune factors present | Solid phase | Apheresis and pooled and ABO | Total = 406<br><br>72.9% of compatible transfusions gave a good response.<br>66.7% of incompatible transfusions gave a poor response.<br><br>Out of 96 transfusions 36 were to alloimmunised patients. | | NS | This study stated that ABO compatibility predicted platelet survival, followed by type, alloimmunisation and clinical factors. This was after investigating these factors and observing platelet counts. They also included platelet counts after transfusion of stored platelet concentrates on days 1, 2, 3, 4 and 5 with the worst count on day 5. |
| Wang et al., 2017 | 204<br>104 females<br>100 males | Haem/Onc patients<br>No mention of immune and non-immune factors.<br><br>114 had HLA/HPA antibodies. | Solid phase for crossmac thing and ELISA for antibody detectio n. | | Total = 480 at 6 per patient.<br><br>310/480 gave increased CCI's | | NS | This study showed that the transfusion of crossmatched platelets to refractory patients could increase the CCI and did not worsen the degree of alloimmunisation. |
| Revelli et al., 2019 | 62 patients | Haem/Onc patients | Capture-P | Apheresis | All patients received a | All transfusions of crossmatch | NS | Retrospective analysis from 2 sites in Italy. One of the sites |

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| | | No mention of immune and non-immune factors.<br><br>All had HLA+/- HPA antibodies. | | | median of 12 and 18 crossmatch compatible platelet transfusions. | compatible platelets gave higher platelet counts than if given random donor platelets. | | did not record some of the data which meant some of it was incomplete.<br>Once refractoriness had been identified no more random donor platelets were issued. |
| Kingsley et al., 2019 | 69 patients | Paediatric oncology patients | Solid phase | ABO matched | 73 platelet crossmatches performed with 41% crossmatch positivity. | 89.2% unsuccessful transfusions showed crossmatch positivity<br>88.9% successful transfusions showed negative crossmatches. | NS | Results indicate that platelet crossmatching may be a reliable tool to select compatible platelet units |
| Chavan et al., 2019 | 38 patients | Haem/Onc patients | Solid phase | ABO matched apheresis | 149 crossmatches performed | 97.8% of crossmatched compatible platelet transfusion episodes showed a satisfactory response. | NS | Platelet crossmatching is an effective intervention in the management of multi-transfused alloimmunised haemato-oncology patients, refractory to platelet transfusion. |
| Desai et al., 2020 | 50 patients | Haematology malignancy patients | Solid phase | ABO matched apheresis | 50 crossmatches | 78% showed compatible and 22% incompatible | NS | Transfusion of crossmatched platelets might provide a small benefit over transfusing |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | results. 18.2% were successful transfusions and 81.8% were unsuccessful in terms of adequate platelet counts. | | randomly selected platelets. |

NS = None specified NPV = Negative predictive value PPV = Positive predictive value Sens = Sensitivity Spec = Specificity Acc = Accuracy

*Table A2: Table showing the patient numbers and characteristics*

| Patient | 001 | 002 | 004 | 006 | 008 | 009 | 010 | 011 |
|---|---|---|---|---|---|---|---|---|
| Age | 64 | 67 | 60 | 76 | 59 | 43 | 64 | 73 |
| Sex M/F | F | M | M | F | M | M | F | M |
| Blood Group | A+ | O+ | O+ | O+ | O+ | A+ | O- | O+ |
| Diagnosis | MF | MF | MF | Lymph | MDS | ALL | ALL | ALL |
| Treatment | ASCT | ASCT | ASCT | Chemo | ASCT | ASCT | Chemo | Chemo |
| Plt Tx | 7 | 4 | 7 | 5 | 29 | 33 | 3 | 4 |

*ALL (Acute Lymphocytic Leukaemia), MDS (Myelodysplasia), Lymph (Lymphoma, MF (Myelofibrosis), ASCT (Allogeneic Stem Cell Transplant), Chemo (Chemotherapy only), Plt Tx (Number of Platelet Transfusions)*

*Table A3: NHSBT Portfolio of blood components and guidance for their clinical use P.55 - Anticoagulants and additive solutions used in components*

| | |
|---|---|
| **CPD**<br>pH 5.3 to 5.9<br>consists of : -<br>Trisodium Citrate (Dihydrate) 26.3 g/L<br>Citric Acid (Monohydrate) 3.27 g/L<br>Sodium Dihydrogen Phosphate (Dihydrate) 2.51g/L<br>Dextrose / Glucose (Monohydrate) 25.5 g/L<br>Water for Injection 1000 mL. | **CPD A1**<br>pH 5.3 to 5.9<br>consists of :-<br>Citric acid.$H_2O$ 3.11- 3.43g/L<br>Sodium Citrate.$2H_2O$ 24.9 - 27.6 g/L<br>Sodium dihydrogen ortho phosphate 2.38 – 2.63 g/L<br>Dextrose. $H_2O$ 30.3 – 33.5 g/L<br>Adenine 331 – 366 mg/L |
| **ACD**<br>pH 4.7 to 5.3<br>consists of : -<br>Sodium Citrate 22.00 g/L<br>Glucose Monohydrate 24.5 g/L<br>Citric Acid (Monohydrate) 8.00 g/L<br>Water for Injection 1000mL | **SAG-M**<br>pH 4.8 to 5.4<br>consists of : -<br>Sodium Chloride 8.77 g/L<br>Dextrose / Glucose Monohydrate 9.00 g/L<br>Adenine 0.169 g/L<br>Mannitol 5.25 g/L<br>Water for Injection 1000mL. |
| **Platelet Additive Solution (1) SSP**<br>pH 7.2<br>consists of : -<br>Sodium Chloride 6.75g/L<br>Sodium Acetate.$3H_2O$ 4.08g/L<br>Sodium Citrate.$2H_2O$ 2.94g/L<br>Water for Injection 1000 mL. | **Additive Solution (2) SSP+ for Pooled Granulocytes and Platelets in Additive Solution and Plasma**<br>pH 7.2<br>consists of : -<br>Sodium Chloride 69.3 mmol/L<br>Sodium Acetate Trihydrate 10.8 mmol/L<br>Sodium Acetate 32.5 mmol/L<br>Sodium Phosphate 28.2 mmol/L<br>Potassium Chloride 5 mmol/L<br>Magnesium Chloride/sulphate 1.5 mmol/L |

*Table A4: Table showing the platelet donation types, their CD62P and C1q results, platelet increments and crossmatch results for patient 001*

| Patient | ABO Match (N/Y) | Aph/Pool | CD62P | C1q | XM Result (C/I) | Successful Tx (Y/N) | Increment |
|---|---|---|---|---|---|---|---|
| 001 | Y | Aph | 824 | 23 | I | N | 2 |
| 001 | Y | Aph | 685 | 45 | I | N | 1 |
| 001 | Y | Aph | 564 | 0 | C | N | 1 |
| 001 | Y | Aph | 134 | 2 | C | Y | 3 |
| 001 | Y | Pool | 217 | 11 | C | Y | 4 |
| 001 | N | Aph | 631 | 13 | C | Y | 4 |
| 001 | Y | Aph | 491 | 21 | C | Y | 3 |

N = No Y = Yes Aph = Apheresis Pool = Pooled Platelet C = compatible I = Incompatible

*Table A5: Table showing the platelet donation types, their CD62P and C1q results, platelet increments and crossmatch results for patient 002*

| Patient | ABO Match (N/Y) | Aph/Pool | CD62P | C1q | XM Result (C/I) | Successful Tx (Y/N) | Increment |
|---|---|---|---|---|---|---|---|
| 002 | Y | Pool† | 171 | 5 | I | | |
| 002 | Y | Pool† | 313 | 12 | I | Y | 10 |
| 002 | Y | Pool | 301 | 3 | C | Y | 4 |
| 002 | Y | Pool | 79 | 8 | C | Y | 6 |
| 002 | N | Pool | 7 | 10 | I | Y | 2 |

† Platelets transfused together and excluded from statistical analysis

*Table A6: Table showing the platelet donation types, their CD62CIP and C1q results, platelet increments and crossmatch results for patient 004*

| Patient | ABO Match (N/Y) | Aph/Pool | CD62P | C1q | XM Result (C/I) | Successful Tx (Y/N) | Increment |
|---|---|---|---|---|---|---|---|
| 004 | N | Aph | 154 | 6 | C | N | 1 |
| 004 | N | Aph | 154 | 6 | C | N | 0 |
| 004 | Y | Pool | 252 | 6 | C | N | 0 |
| 004 | N | Aph | 504 | 16 | C | N | 1 |
| 004 | Y | Pool | 205 | 11 | C | N | 0 |
| 004 | N | Aph | 144 | 20 | C | N | 1 |
| 004 | N | Pool | 140 | 11 | C | N | 0 |

*Table A7: Table showing the platelet donation types, their CD62P and C1q results, platelet increments and crossmatch results for patient 006*

| Patient | ABO Match (N/Y) | Aph/Pool | CD62P | C1q | XM Result (C/I) | Successful Tx (Y/N) | Increment |
|---|---|---|---|---|---|---|---|
| 006 | Y | Pool | 326 | 16 | C | N | 1 |
| 006 | Y | Pool | 697 | 10 | I | N | 0 |
| 006 | Y | Pool | 238 | 5 | C | N | 1 |
| 006 | Y | Pool | 477 | 14 | C | Y | 2 |
| 006 | Y | Pool | 643 | 11 | I | Y | 7 |

*Table A8: Table showing the platelet donation types, their CD62P and C1q results, platelet increments and crossmatch results for patient 008*

| Patient | ABO Match (N/Y) | Aph/Pool | CD62P | C1q | XM Result (C/I) | Successful Tx (Y/N) | Increment |
|---|---|---|---|---|---|---|---|
| 008 | Y | Pool | 226 | 27 | I | N | 1 |
| 008 | Y | Pool | 386 | 8 | I | Y | 9 |
| 008 | Y | Pool | 552 | 5 | I | N | -3 |
| 008 | Y | Pool | 174 | 13 | I | N | 3 |
| 008 | Y | Pool | 224 | 12 | C | N | 2 |
| 008 | N | Pool | 396 | 15 | I | N | -1 |
| 008 | Y | Aph | 823 | 13 | C | Y | 9 |
| 008 | N | Aph | 770 | 41 | I | N | -1 |
| 008 | Y | Aph | 546 | 15 | C | Y | 6 |
| 008 | Y | Aph | 200 | 7 | I | N | 1 |
| 008 | Y | Pool | 155 | 18 | C | Y | 4 |
| 008 | Y | Pool | 247 | 29 | I | Y | 9 |
| 008 | Y | Pool | 172 | 13 | I | Y | 9 |
| 008 | Y | Aph | 647 | 13 | C | N | -11 |
| 008 | Y | Aph | 387 | 25 | I | N | -1 |
| 008 | Y | Pool | 451 | 20 | I | Y | 5 |
| 008 | Y | Pool† | 385 | 17 | I | | |
| 008 | N | Pool† | 260 | 14 | I | Y | 5 |
| 008 | Y | Pool† | 327 | 21 | I | | |
| 008 | Y | Pool† | 235 | 16 | I | N | -8 |
| 008 | Y | Pool† | 154 | 26 | C‡ | | |
| 008 | N | Pool† | 616 | 22 | I‡ | N | 2 |
| 008 | Y | Pool† | 480 | 29 | I‡ | | |
| 008 | N | Pool† | 1013 | 18 | C‡ | Y | 5 |
| 008 | Y | Pool | 220 | 20 | I | Y | 3 |
| 008 | Y | Pool | 139 | 22 | I | Y | 0 |
| 008 | Y | Pool | 120 | 22 | I | Y | 2 |
| 008 | y | Pool | 147 | 16 | I | Y | 0 |
| 008 | Y | Pool† | 533 | 17 | I | | |
| 008 | Y | Pool† | 380 | 20 | I | | |
| 008 | Y | Pool† | 151 | 15 | I | Y | 4 |

†Platelets transfused together and excluded from statistical analysis

‡ Removed from calculations as there was one compatible and one incompatible unit for a double transfusion.

*Table A9: Table showing the platelet donation types, their CD62P and C1q results, platelet increments and crossmatch results for patient 009*

| Patient | ABO Match (N/Y) | Aph/Pool | CD62P | C1q | XM Result (C/I) | Successful Tx (Y/N) | Increment |
|---|---|---|---|---|---|---|---|
| 009 | Y | Pool | 618 | 15 | C | Y | 4 |
| 009 | Y | Pool | 139 | 21 | C | Y | 9 |
| 009 | Y | Pool | 243 | 5 | C | Y | 14 |
| 009 | Y | Aph | 117 | 4 | C | N | -5 |
| 009 | Y | Aph | 725 | 18 | I | Y | 7 |
| 009 | Y | Pool | 640 | 7 | C | Y | 0 |
| 009 | Y | Pool | 285 | 5 | C | Y | 2 |
| 009 | Y | Aph | 970 | 15 | I | Y | 4 |
| 009 | Y | Aph† | 903 | 5 | C | | |
| 009 | Y | Aph† | 767 | 16 | I | Y | 13 |
| 009 | Y | Aph | 817 | 68 | I | Y | 9 |
| 009 | Y | Pool | 217 | 11 | I | Y | 8 |
| 009 | N | Pool | 182 | 14 | I | Y | 5 |
| 009 | Y | Pool | 125 | 15 | C | Y | 6 |
| 009 | Y | Aph | 125 | 15 | I | Y | 12 |
| 009 | Y | Aph | 1043 | 11 | I | Y | 4 |
| 009 | Y | Pool | 889 | 24 | I | Y | 6 |
| 009 | N | Aph | 270 | 10 | I | Y | 6 |
| 009 | Y | Aph | 1020 | 57 | C | Y | 8 |
| 009 | Y | Pool | 640 | 27 | I | Y | 8 |
| 009 | Y | Aph | 369 | 13 | I | N | -2 |
| 009 | Y | Aph | 1140 | 28 | C | Y | 0 |
| 009 | Y | Pool | 355 | 22 | I | Y | 10 |
| 009 | Y | Pool | 446 | 59 | I | N | -5 |
| 009 | Y | Aph | 263 | 31 | C | Y | 1 |
| 009 | Y | Pool | 169 | 16 | C | N | -1 |
| 009 | Y | Pool | 509 | 11 | C | N | -2 |
| 009 | Y | Aph | 276 | 12 | C | Y | 3 |
| 009 | Y | Pool | 379 | 15 | I | Y | 2 |
| 009 | Y | Pool | 296 | 12 | I | Y | 0 |
| 009 | Y | Pool | 470 | 20 | C | Y | 5 |
| 009 | Y | Aph | 396 | 18 | C | Y | 4 |
| 009 | Y | Aph | 208 | 26 | C | Y | 6 |
| 009 | Y | Aph | 720 | 22 | C | Y | 7 |

†Platelets transfused together and excluded from statistical analysis

*Table A10: Table showing the platelet donation types, their CD62P and C1q results, platelet increments and crossmatch results for patient 010*

| Patient | ABO Match (N/Y) | Aph/Pool | CD62P | C1q | XM Result (C/I) | Successful Tx (Y/N) | Increment |
|---|---|---|---|---|---|---|---|
| 010 | Y | Aph | 793 | 5 | C | N | 2 |
| 010 | N | Pool | 132 | 8 | C | N | 0 |
| 010 | Y | Pool | 134 | 18 | I | N | No result |

*Table A11: Table showing the platelet donation types, their CD62P and C1q results, platelet increments and crossmatch results for patient 011*

| Patient | ABO Match (N/Y) | Aph/Pool | CD62P | C1q | XM Result (C/I) | Successful Tx (Y/N) | Increment |
|---|---|---|---|---|---|---|---|
| 011 | Y | Pool | 498 | 5 | C | N | 2 |
| 011 | N | Aph | 578 | 33 | I | N | 1 |
| 011 | N | Aph | 540 | 36 | I | Y | 9 |
| 011 | Y | Pool | 246 | 37 | I | Y | 11 |

*Table A12: Median Fluorescence Intensities (MFI) for each of the markers CD62P, C1q, C3, IgG and C3dg and mean platelet volumes (MPV) in 20 normal subjects, allowing a mean and SD to be determined for each.*

| | | Platelet Marker | | | | |
|---|---|---|---|---|---|---|
| | **MPV** | **CD62P** | **C1q** | **C3** | **IgG** | **C3dg** |
| | 8.6 | 140 | 11 | 9 | 21 | 0 |
| | 6.8 | 237 | 10 | 7 | 17 | 9 |
| | 8.1 | 150 | 12 | 7 | 13 | 10 |
| | 8.3 | 325 | 8 | 6 | 14 | 11 |
| | 8 | 94 | 7 | 13 | 23 | 3 |
| | 7.6 | 203 | 10 | 7 | 25 | 3 |
| | 6.4 | 170 | 10 | 14 | 23 | 9 |
| | 7.7 | 279 | 12 | 9 | 7 | 4 |
| | 8 | 218 | 7 | 9 | 11 | 5 |
| | 6.8 | 280 | 13 | 11 | 14 | 8 |
| | 8.4 | 177 | 7 | 11 | 15 | 10 |
| | 9.1 | 211 | 11 | 10 | 13 | 5 |
| | 7.1 | 174 | 6 | 9 | 10 | 12 |
| | 7.1 | 153 | 10 | 12 | 9 | 10 |
| | 7.6 | 185 | 9 | 11 | 15 | 9 |
| | 7.8 | 165 | 12 | 9 | 12 | 8 |
| | 6.6 | 146 | 15 | 8 | 14 | 6 |
| | 6.7 | 214 | 13 | 15 | 16 | 4 |
| | 6.9 | 231 | 10 | 13 | 12 | 15 |
| | 7 | 196 | 17 | 10 | 11 | 9 |
| Mean | **7.5** | **197.4** | **10.5** | **10** | **14.8** | **7.5** |
| SD† | **0.75** | **54.8** | **2.8** | **2.5** | **4.9** | **3.6** |

†SD = Standard Deviation

*Table A13: Tests for normality using the Kolmogorov-Smirnov test for CD62P, C1q, C3, IgG, C3dg and MPV in 20 normal patients showing that the results represent a normal distribution*

Tabular results

| hd Lognormality Tests<br>abular results | A<br>CD62P | B<br>C1q | C<br>C3 | D<br>IgG | E<br>C3dg | F<br>MPV |
|---|---|---|---|---|---|---|
| **Test for normal distribution** | | | | | | |
| **Kolmogorov-Smirnov test** | | | | | | |
| KS distance | 0.1036 | 0.1291 | 0.1559 | 0.1796 | 0.1595 | 0.1659 |
| P value | >0.1000 | >0.1000 | >0.1000 | 0.0903 | >0.1000 | >0.1000 |
| Passed normality test (alpha=0.05)' | Yes | Yes | Yes | Yes | Yes | Yes |
| P value summary | ns | ns | ns | ns | ns | ns |
| | | | | | | |
| **Number of values** | 20 | 20 | 20 | 20 | 20 | 20 |

*Table A14: Median Fluorescence Intensities (MFI) for each of the markers CD62P, C1q, C3, IgG and C3dg in 51 apheresis platelet donations, allowing a median and range to be determined for each.*

| | | Platelet Marker | | | | |
|---|---|---|---|---|---|---|
| Unit | **MPV (fL)** | **CD62P** | **C1q (MFI)** | **C3 (MFI)** | **IgG (MFI)** | **C3dg (MFI)** |
| 1 | 5.4 | 436 | 68 | 9 | 7 | 2 |
| 2 | 5.9 | 338 | 54 | 12 | 6 | 8 |
| 3 | 3.6 | 1158 | 298 | 13 | 4 | 7 |
| 4 | 4.2 | 1059 | 287 | 15 | 5 | 9 |
| 5 | 6.2 | 323 | 16 | 11 | 3 | 10 |
| 6 | 4.6 | 207 | 12 | 9 | 8 | 11 |
| 7 | 3.9 | 904 | 168 | 9 | 9 | 1 |
| 8 | 4 | 1150 | 234 | 8 | 10 | 3 |
| 9 | 5.6 | 439 | 69 | 9 | 6 | 7 |
| 10 | 5.2 | 439 | 87 | 10 | 8 | 6 |
| 11 | 5.8 | 232 | 12 | 12 | 3 | 12 |
| 12 | 5.4 | 111 | 11 | 13 | 9 | 14 |
| 13 | 5 | 314 | 13 | 16 | 7 | 13 |
| 14 | 4.8 | 844 | 268 | 15 | 9 | 10 |
| 15 | 5.4 | 194 | 16 | 9 | 3 | 4 |
| 16 | 4.6 | 304 | 54 | 9 | 4 | 5 |
| 17 | 5.3 | 165 | 17 | 8 | 10 | 7 |
| 18 | 4.4 | 527 | 261 | 12 | 6 | 9 |
| 19 | 3.9 | 641 | 182 | 11 | 8 | 8 |
| 20 | 4.1 | 1212 | 367 | 14 | 6 | 8 |
| 21 | 5.1 | 459 | 119 | 15 | 9 | 6 |
| 22 | 6.1 | 347 | 18 | 11 | 3 | 4 |
| 23 | 5 | 377 | 19 | 10 | 5 | 7 |
| 24 | 5.8 | 629 | 88 | 11 | 4 | 8 |
| 25 | 4.9 | 509 | 68 | 14 | 6 | 9 |
| 26 | 3.7 | 990 | 395 | 15 | 6 | 10 |
| 27 | 3.8 | 803 | 234 | 15 | 5 | 15 |
| 28 | 5.8 | 217 | 67 | 10 | 7 | 16 |
| 29 | 3.4 | 1084 | 256 | 16 | 3 | 16 |
| 30 | 4.8 | 348 | 65 | 15 | 4 | 15 |
| 31 | 4.7 | 483 | 98 | 12 | 2 | 11 |
| 32 | 7.5 | 551 | 185 | 11 | 5 | 17 |
| 33 | 6.4 | 366 | 85 | 17 | 2 | 13 |
| 34 | 3.7 | 535 | 98 | 18 | 6 | 17 |
| 35 | 5.5 | 222 | 62 | 11 | 3 | 6 |
| 36 | 5.1 | 425 | 162 | 11 | 6 | 5 |
| 37 | 5.4 | 102 | 110 | 10 | 8 | 6 |
| 38 | 5.7 | 246 | 35 | 8 | 9 | 14 |
| 39 | 5 | 328 | 160 | 10 | 7 | 12 |
| 40 | 5.4 | 433 | 181 | 8 | 6 | 13 |
| 41 | 4.8 | 1404 | 469 | 16 | 4 | 14 |
| 42 | 3.6 | 969 | 365 | 13 | 8 | 13 |
| 43 | 4.2 | 1437 | 372 | 14 | 6 | 12 |
| 44 | 4.7 | 269 | 92 | 6 | 9 | 5 |
| 45 | 4.5 | 576 | 217 | 10 | 5 | 12 |
| 46 | 3.8 | 1189 | 242 | 10 | 7 | 13 |
| 47 | 5.3 | 793 | 70 | 15 | 6 | 8 |
| 48 | 5.1 | 587 | 18 | 9 | 3 | 19 |
| 49 | 3.4 | 1150 | 235 | 14 | 8 | 22 |
| 50 | 4.7 | 822 | 168 | 17 | 4 | 24 |
| 51 | 5.3 | 262 | 46 | 14 | 6 | 12 |
| Median | 5 | 459 | 98 | 11 | 6 | 10 |
| Range | 4.1 | 1335 | 458 | 12 | 8 | 22 |

*Table A15: Tests for normality using the Kolmogorov-Smirnov test for CD62P, C1q, C3, IgG and C3dg in 51 apheresis platelet donations showing that the results do not represent a normal distribution*

| Normality and Lognormality Tests<br>Tabular results | A<br>CD62P | B<br>C1q | C<br>C3 | D<br>IgG | E<br>C3dg | F<br>MPV |
|---|---|---|---|---|---|---|
| **Test for normal distribution** | | | | | | |
| **Kolmogorov-Smirnov test** | | | | | | |
| KS distance | 0.1481 | 0.1761 | 0.1400 | 0.1186 | 0.09529 | 0.07050 |
| P value | 0.0070 | 0.0004 | 0.0140 | 0.0706 | >0.1000 | >0.1000 |
| Passed normality test (alpha=0.05)' | No | No | No | Yes | Yes | Yes |
| P value summary | ** | *** | * | ns | ns | ns |
| | | | | | | |
| **Number of values** | 51 | 51 | 51 | 51 | 51 | 51 |

*Table A16: Median Fluorescence Intensities (MFI) for each of the markers CD62P, C1q, C3, IgG and C3dg in 51 pooled platelet donations, allowing a median and range to be determined for each.*

| | | Platelet Marker | | | | |
|---|---|---|---|---|---|---|
| Unit | **MPV (fL)** | **CD62P (MFI)** | **C1q (MFI)** | **C3 (MFI)** | **IgG (MFI)** | **C3dg (MFI)** |
| 1 | 4.8 | 296 | 12 | 8 | 6 | 11 |
| 2 | 5.8 | 343 | 23 | 10 | 2 | 15 |
| 3 | 5.7 | 279 | 24 | 7 | 3 | 13 |
| 4 | 5.6 | 392 | 135 | 8 | 5 | 14 |
| 5 | 5.6 | 279 | 19 | 9 | 4 | 12 |
| 6 | 5.7 | 300 | 21 | 5 | 6 | 12 |
| 7 | 5.8 | 208 | 15 | 8 | 8 | 7 |
| 8 | 6 | 140 | 18 | 5 | 7 | 9 |
| 9 | 5.3 | 351 | 24 | 8 | 12 | 10 |
| 10 | 5.4 | 175 | 25 | 9 | 10 | 13 |
| 11 | 6.3 | 198 | 32 | 7 | 9 | 12 |
| 12 | 5.5 | 232 | 24 | 8 | 6 | 14 |
| 13 | 6.2 | 175 | 27 | 6 | 7 | 17 |
| 14 | 6.4 | 212 | 22 | 4 | 5 | 16 |
| 15 | 5.6 | 169 | 19 | 8 | 3 | 12 |
| 16 | 5.5 | 92 | 45 | 9 | 1 | 13 |
| 17 | 5.8 | 154 | 21 | 10 | 2 | 10 |
| 18 | 6.4 | 265 | 22 | 9 | 8 | 10 |
| 19 | 6.8 | 128 | 25 | 11 | 9 | 9 |
| 20 | 6.8 | 68 | 22 | 12 | 1 | 9 |
| 21 | 7.9 | 56 | 46 | 9 | 5 | 4 |
| 22 | 6.6 | 407 | 218 | 12 | 6 | 12 |
| 23 | 6.9 | 428 | 107 | 9 | 6 | 11 |
| 24 | 7.1 | 256 | 43 | 7 | 7 | 12 |
| 25 | 6.4 | 375 | 121 | 10 | 3 | 12 |
| 26 | 5.5 | 410 | 241 | 10 | 13 | 15 |
| 27 | 6.7 | 293 | 59 | 9 | 12 | 16 |
| 28 | 5.7 | 405 | 295 | 12 | 1 | 14 |
| 29 | 6.1 | 207 | 36 | 12 | 8 | 13 |
| 30 | 6 | 270 | 48 | 15 | 6 | 14 |
| 31 | 6 | 367 | 83 | 8 | 7 | 13 |
| 32 | 4.9 | 227 | 49 | 7 | 7 | 19 |
| 33 | 6.2 | 211 | 62 | 6 | 8 | 15 |
| 34 | 6.3 | 208 | 45 | 7 | 3 | 11 |
| 35 | 7.1 | 89 | 27 | 6 | 4 | 10 |
| 36 | 5.7 | 198 | 21 | 15 | 5 | 14 |
| 37 | 6.1 | 271 | 21 | 14 | 6 | 15 |
| 38 | 5.8 | 314 | 24 | 12 | 4 | 9 |
| 39 | 5.9 | 287 | 21 | 12 | 6 | 8 |
| 40 | 6.7 | 355 | 44 | 16 | 5 | 14 |
| 41 | 6.3 | 146 | 19 | 13 | 2 | 12 |
| 42 | 6.2 | 292 | 22 | 12 | 4 | 14 |
| 43 | 7.8 | 344 | 33 | 14 | 3 | 11 |
| 44 | 7.3 | 287 | 23 | 15 | 8 | 11 |
| 45 | 5.6 | 240 | 24 | 11 | 5 | 13 |
| 46 | 4.5 | 265 | 20 | 9 | 4 | 14 |
| 47 | 5.3 | 181 | 20 | 8 | 6 | 13 |
| 48 | 5.5 | 216 | 21 | 7 | 7 | 14 |
| 49 | 6.1 | 248 | 38 | 9 | 5 | 17 |
| 50 | 5.8 | 247 | 41 | 6 | 2 | 9 |
| 51 | 6.5 | 245 | 39 | 14 | 4 | 12 |
| Median | 6 | 248 | 25 | 9 | 6 | 12 |
| Range | 3.4 | 372 | 283 | 12 | 11 | 15 |

*Table A17: Tests for normality using the Kolmogorov-Smirnov test for CD62P, C1q, C3, IgG and C3dg in 51 pooled platelet donations showing that the results do not represent a normal distribution*

| Normality and Lognormality Tests<br>Tabular results | A<br>CD62P | B<br>C1q | C<br>C3 | D<br>IgG | E<br>C3dg | F<br>MPV |
|---|---|---|---|---|---|---|
| **Test for normal distribution** | | | | | | |
| **Kolmogorov-Smirnov test** | | | | | | |
| KS distance | 0.06100 | 0.3218 | 0.1623 | 0.1107 | 0.1182 | 0.1026 |
| P value | >0.1000 | <0.0001 | 0.0018 | >0.1000 | 0.0722 | >0.1000 |
| Passed normality test (alpha=0.05)' | Yes | No | No | Yes | Yes | Yes |
| P value summary | ns | **** | ** | ns | ns | ns |
| | | | | | | |
| **Number of values** | 51 | 51 | 51 | 51 | 51 | 51 |

*Table A18: Results using Spearman's rank correlation coefficient for apheresis platelet donations due to the non-parametric distribution of the data showing the relationship between the MPV and CD62P.*

| Correlation<br>Tabular results | A<br>MPV<br>vs.<br>CD62P |
|---|---|
| **Spearman r** | |
| r | -0.6555 |
| 95% confidence interval | -0.7918 to -0.4571 |
| | |
| **P value** | |
| P (two-tailed) | <0.0001 |
| P value summary | **** |
| Exact or approximate P value' | Approximate |
| Significant? (alpha = 0.05) | Yes |
| | |
| **Number of XY Pairs** | 51 |
| | |

*Table A19: Results using Spearman's rank correlation coefficient for pooled platelet donations due to the non-parametric distribution of the data showing the relationship between the MPV and CD62P.*

| | Correlation<br>Tabular results | MPV<br>vs.<br>CD62P |
|---|---|---|
| 1 | **Spearman r** | |
| 2 | r | -0.05292 |
| 3 | 95% confidence interval | -0.3313 to 0.2339 |
| 4 | | |
| 5 | **P value** | |
| 6 | P (two-tailed) | 0.7122 |
| 7 | P value summary | ns |
| 8 | Exact or approximate P value' | Approximate |
| 9 | Significant? (alpha = 0.05) | No |
| 10 | | |
| 11 | **Number of XY Pairs** | 51 |
| 12 | | |

*Table A20: The effects of platelet transfusion on platelet count, MPV and platelet markers for patient 001*

| Date of Transfusion | Sample Date | Platelet Count | MPV | CD62P | C1q | IgG | C3 | C3dg |
|---|---|---|---|---|---|---|---|---|
| 25/09/2020 | 25/09/2020 | 8 | 9.2 | 1791 | 643 | 12 | 7 | 7 |
| | 25/09/2020 | 10 | 10.3 | 765 | 565 | 7 | 8 | 8 |
| 28/09/2020 | 28/09/2020 | 8 | 7.9 | 1029 | 499 | 17 | 11 | 15 |
| 30/09/2020 | 30/09/2020 | 9 | 10.4 | 972 | 725 | 26 | 10 | 10 |
| 02/10/2020 | 02/10/2020 | 10 | 9.5 | 1243 | 635 | 19 | 7 | 4 |
| | 03/10/2020 | 13 | 9.6 | 547 | 578 | 20 | 6 | 5 |
| 05/10/2020 | 05/10/2020 | 8 | 10 | 1177 | 752 | 15 | 8 | 8 |
| | 07/10/2020 | 12 | 9.7 | 1366 | 200 | 13 | 9 | 9 |
| 09/11/2020 | 09/11/2020 | 8 | 9.9 | 3284 | 214 | 9 | 10 | 10 |
| | 11/11/2020 | 12 | 10.7 | 1100 | 201 | 14 | 9 | 12 |
| 18/11/2020 | 18/11/2020 | 10 | 11.3 | 1204 | 270 | 12 | 8 | 8 |

*Table A21: The effects of platelet transfusion on platelet count, MPV and platelet markers for patient 002*

| Date of Transfusion | Sample Date | Platelet Count | MPV | CD62P | C1q | IgG | C3 | C3dg |
|---|---|---|---|---|---|---|---|---|
| 23/09/2020 | 23/09/2020 | 5 | 5.8 | 457 | 20 | 7 | 12 | 15 |
| | 24/09/2020 | 15 | 8.6 | 1253 | 12 | 10 | 13 | 17 |
| 05/10/2020 | 05/10/2020 | 8 | 6.7 | 1037 | 15 | 9 | 10 | 13 |
| | 07/10/2020 | 12 | 7.4 | 180 | 18 | 8 | 11 | 18 |
| 09/10/2020 | 09/10/2020 | 8 | 7.2 | 401 | 14 | 3 | 10 | 16 |
| | 12/10/2020 | 14 | 6.9 | 354 | 15 | 5 | 9 | 14 |
| 16/10/2020 | 16/10/2020 | 9 | 6.3 | 247 | 27 | 6 | 12 | 16 |

*Table A22: The effects of platelet transfusion on platelet count, MPV and platelet markers for patient 004*

| Date of Transfusion | Sample Date | Platelet Count | MPV | CD62P | C1q | IgG | C3 | C3dg |
|---|---|---|---|---|---|---|---|---|
| 02/10/2020 | 02/10/2020 | 4 | 9.2 | 549 | 44 | 10 | 10 | 13 |
| 03/10/2020 | 03/10/2020 | 5 | 7.1 | 246 | 24 | 8 | 8 | 15 |
| | 04/10/2020 | 5 | 7.9 | 603 | 37 | 7 | 9 | 12 |
| 05/10/2020 | 05/10/2020 | 3 | 8.8 | 185 | 32 | 9 | 7 | 17 |
| 06/10/2020 | 06/10/2020 | 3 | 9.3 | 77 | 18 | 12 | 8 | 16 |
| 07/10/2020 | 07/10/2020 | 4 | 7.9 | 858 | 59 | 10 | 9 | 12 |
| 08/10/2020 | 08/10/2020 | 4 | 9.5 | 143 | 43 | 9 | 10 | 18 |
| 09/10/2020 | 09/10/2020 | 5 | 8.8 | 122 | 23 | 8 | 8 | 14 |

*Table A23: The effects of platelet transfusion on platelet count, MPV and platelet markers for patient 006*

| Date of Transfusion | Sample Date | Platelet Count | MPV | CD62P | C1q | IgG | C3 | C3dg |
|---|---|---|---|---|---|---|---|---|
| 20/10/2020 | 20/10/2020 | 7 | 7.3 | 187 | 14 | 10 | 12 | 17 |
| 21/10/2020 | 21/10/2020 | 8 | 7.9 | 67 | 17 | 8 | 14 | 16 |
| 22/10/2020 | 22/10/2020 | 8 | 8 | 132 | 48 | 9 | 13 | 18 |
| 23/10/2020 | 23/10/2020 | 9 | 8.8 | 252 | 11 | 8 | 15 | 16 |
| | 24/10/2020 | 11 | 8.4 | 189 | 13 | 9 | 12 | 19 |
| 25/10/2020 | 25/10/2020 | 7 | 6.3 | 849 | 48 | 10 | 10 | 17 |

*Table A24: The effects of platelet transfusion on platelet count, MPV and platelet markers for patient 008*

| Date of Transfusion | Sample Date | Platelet Count | MPV | CD62P | C1q | IgG | C3 | C3dg |
|---|---|---|---|---|---|---|---|---|
| 28/10/2020 | 28/10/2020 | 8 | 9.2 | 662 | 91 | 12 | 7 | 7 |
| | 30/10/2020 | 10 | 10.3 | 702 | 15 | 7 | 8 | 8 |
| 05/11/2020 | 05/11/2020 | 8 | 7.9 | 790 | 12 | 17 | 11 | 15 |
| | 06/11/2020 | 9 | 10.4 | 199 | 8 | 26 | 10 | 10 |
| 12/11/2020 | 12/11/2020 | 10 | 9.5 | 848 | 16 | 19 | 7 | 4 |
| 16/11/2020 | 16/11/2020 | 13 | 9.6 | 505 | 17 | 20 | 6 | 5 |
| 19/11/2020 | 19/11/2020 | 8 | 10 | 1099 | 29 | 21 | 8 | 10 |
| 25/11/2020 | 25/11/2020 | 12 | 9.7 | 1361 | 20 | 20 | 6 | 12 |
| 30/11/2020 | 30/11/2020 | 8 | 9.9 | 1381 | 38 | 23 | 7 | 10 |
| 03/12/2020 | 03/12/2020 | 10 | 11.3 | 985 | 28 | 19 | 9 | 14 |
| 07/12/2020 | 07/12/2020 | 13 | 10.7 | 992 | 16 | 21 | 10 | 11 |
| 10/12/2020 | 10/12/2020 | 5 | 8.1 | 300 | 21 | 20 | 11 | 14 |
| 11/12/2020 | 11/12/2020 | 6 | 8.4 | 335 | 20 | 18 | 13 | 15 |
| 14/12/2020 | 14/12/2020 | 4 | 6.8 | 1141 | 27 | 13 | 9 | 10 |
| 16/12/2020 | 16/12/2020 | 8 | 6.6 | 694 | 18 | 14 | 8 | 12 |
| 17/12/2020 | 17/12/2020 | 17 | 6.3 | 940 | 16 | 18 | 5 | 14 |
| 18/12/2020 | 18/12/2020 | 6 | 7.1 | 475 | 20 | 17 | 7 | 17 |
| 19/12/2020 | 19/12/2020 | 5 | 7.6 | 1571 | 33 | 20 | 6 | 9 |
| 20/12/2020 | 20/12/2020 | 10 | 7.3 | 1557 | 26 | 24 | 8 | 10 |
| 21/12/2020 | 21/12/2020 | 15 | 7.2 | 1308 | 12 | 20 | 7 | 8 |
| 22/12/2020 | 22/12/2020 | 7 | 6.7 | 159 | 15 | 26 | 9 | 10 |
| 23/12/2020 | 23/12/2020 | 9 | 7.6 | 924 | 22 | 25 | 8 | 9 |
| 24/12/2020 | 24/12/2020 | 14 | 7.9 | 295 | 18 | 20 | 9 | 8 |
| 25/12/2020 | 25/12/2020 | 17 | 7.5 | 56 | 17 | 24 | 10 | 11 |
| 26/12/2020 | 26/12/2020 | 17 | 7.9 | 145 | 17 | 7 | 11 | 9 |
| 27/12/2020 | 27/12/2020 | 19 | 7.9 | 353 | 22 | 9 | 9 | 10 |
| 29/12/2020 | 29/12/2020 | 16 | 6.8 | 1747 | 26 | 15 | 8 | 8 |

*Table A25: The effects of platelet transfusion on platelet count, MPV and platelet markers for patient 009*

| Date of Transfusion | Sample Date | Platelet Count | MPV | CD62P | C1q | IgG | C3 | C3dg |
|---|---|---|---|---|---|---|---|---|
| 26/10/2020 | 26/10/2020 | 8 | 2.9 | 324 | 32 | 10 | 9 | 9 |
| 29/10/2020 | 29/10/2020 | 9 | 4 | 196 | 12 | 9 | 8 | 8 |
| 04/11/2020 | 04/11/2020 | 5 | 6 | 425 | 21 | 13 | 9 | 6 |
| | 05/11/2020 | 19 | 6.2 | 341 | 4 | 17 | 10 | 5 |
| 06/11/2020 | 06/11/2020 | 8 | 7.5 | 154 | 6 | 16 | 11 | 7 |
| 09/11/2020 | 09/11/2020 | 3 | 5.8 | 58 | 4 | 14 | 9 | 12 |
| 11/11/2020 | 11/11/2020 | 10 | 5.3 | 951 | 25 | 15 | 10 | 11 |
| 12/11/2020 | 12/11/2020 | 10 | 7.3 | 679 | 18 | 13 | 19 | 13 |
| 13/11/2020 | 13/11/2020 | 12 | 7.6 | 1106 | 15 | 15 | 12 | 18 |
| 14/11/2020 | 14/11/2020 | 16 | 7 | 344 | 16 | 17 | 14 | 13 |
| 18/11/2020 | 18/11/2020 | 4 | 7 | 1061 | 26 | 16 | 9 | 14 |
| | 19/11/2020 | 13 | 8 | 390 | 22 | 19 | 6 | 13 |
| 20/11/2020 | 20/11/2020 | 8 | 7.6 | 205 | 18 | 10 | 7 | 15 |
| | 21/11/2020 | 16 | 7 | 109 | 24 | 13 | 12 | 15 |
| 23/11/2020 | 23/11/2020 | 6 | 7.7 | 273 | 12 | 12 | 10 | 16 |
| 24/11/2020 | 24/11/2020 | 11 | 7.1 | 109 | 9 | 14 | 9 | 11 |
| 28/11/2020 | 28/11/2020 | 5 | 6.9 | 137 | 13 | 15 | 8 | 12 |
| 30/11/2020 | 30/11/2020 | 5 | 6.1 | 358 | 15 | 11 | 10 | 17 |
| 01/12/2020 | 01/12/2020 | 9 | 7.6 | 177 | 16 | 17 | 15 | 14 |
| 04/12/2020 | 04/12/2020 | 8 | 4.4 | 327 | 11 | 16 | 18 | 15 |
| 05/12/2020 | 05/12/2020 | 14 | 5.6 | 166 | 7 | 15 | 9 | 15 |
| 09/12/2020 | 09/12/2020 | 12 | 6.7 | 258 | 12 | 12 | 8 | 9 |
| 10/12/2020 | 10/12/2020 | 20 | 7.4 | 155 | 11 | 14 | 13 | 8 |
| 11/12/2020 | 11/12/2020 | 18 | 8.1 | 373 | 11 | 15 | 6 | 9 |
| 13/12/2020 | 13/12/2020 | 10 | 7.4 | 530 | 13 | 13 | 7 | 12 |
| 14/12/2020 | 14/12/2020 | 20 | 7.1 | 591 | 12 | 15 | 9 | 15 |
| 15/12/2020 | 15/12/2020 | 15 | 8 | 437 | 13 | 18 | 12 | 13 |
| 16/12/2020 | 16/12/2020 | 16 | 7.9 | 1395 | 15 | 16 | 14 | 18 |
| 17/12/2020 | 17/12/2020 | 15 | 8.8 | 1018 | 20 | 14 | 13 | 19 |
| 18/12/2020 | 18/12/2020 | 13 | 9 | 581 | 20 | 13 | 14 | 13 |
| 19/12/2020 | 19/12/2020 | 16 | 9.4 | 1680 | 34 | 14 | 16 | 14 |
| 20/12/2020 | 20/12/2020 | 18 | 9.5 | 1441 | 23 | 13 | 18 | 13 |
| 21/12/2020 | 21/12/2020 | 18 | 6.5 | 873 | 22 | 19 | 13 | 14 |
| 22/12/2020 | 22/12/2020 | 23 | 5.1 | 212 | 12 | 18 | 18 | 15 |
| 23/12/2020 | 23/12/2020 | 27 | 5.6 | 717 | 18 | 15 | 13 | 13 |
| 25/12/2020 | 25/12/2020 | 29 | 7.2 | 268 | 8 | 16 | 10 | 18 |

*Table A26: The effects of platelet transfusion on platelet count, MPV and platelet markers for patient 010*

| Date of Transfusion | Sample Date | Platelet Count | MPV | CD62P | C1q | IgG | C3 | C3dg |
|---|---|---|---|---|---|---|---|---|
| 28/11/2020 | 28/11/2020 | 6 | 4.4 | 292 | 13 | 10 | 9 | 9 |
| 01/12/2020 | 01/12/2020 | 3 | 4.6 | 174 | 54 | 11 | 8 | 10 |
| 09/12/2020 | 08/12/2020 | 4 | 3.9 | 161 | 8 | 13 | 7 | 13 |

*Table A27: The effects of platelet transfusion on platelet count, MPV and platelet markers for patient 011*

| Date of Transfusion | Sample Date | Platelet Count | MPV | CD62P | C1q | IgG | C3 | C3dg |
|---|---|---|---|---|---|---|---|---|
| 30/11/2020 | 30/11/2020 | 6 | 7.4 | 192 | 12 | 13 | 9 | 96 |
| 02/12/2020 | 02/12/2020 | 8 | 7.4 | 472 | 9 | 9 | 7 | 10 |
| 04/12/2020 | 04/12/2020 | 7 | 6.3 | 418 | 19 | 10 | 6 | 15 |
| 09/12/2020 | 09/12/2020 | 78 | 7.3 | 647 | 8 | 13 | 9 | 13 |
| | 10/12/2020 | 89 | 6.9 | 389 | 6 | 14 | 5 | 11 |

*Table A28: Apheresis platelet donations transfused to patients showing whether they were compatible or incompatible and their CD62P and C1q values, those in red represent values above the normal range.*

| Platelet | MPV | CD62P | C1q | C/I |
|---|---|---|---|---|
| 533 5 Aph A- | 6.6 | 824 | 23 | I |
| 449 N Aph A- | 5.1 | 685 | 45 | I |
| 542 Z Aph A+ | 5.8 | 564 | 0 | C |
| 835 E Aph A+ | 6.7 | 134 | 2 | C |
| 722 6 O+ Aph | 5.8 | 631 | 13 | C |
| 576 * A+ Aph | 7.6 | 491 | 21 | C |
| 319 2 Aph A+ Pk1 | 7.4 | 154 | 6 | |
| 319 2 Aph A+ Pk2 | 7.4 | 154 | 6 | C |
| 835 3 Aph A- | 6.6 | 504 | 16 | C |
| 067 L Aph A- | 6.2 | 144 | 20 | C |
| 716 3 O- Aph | 5.8 | 823 | 13 | C |
| 794 C A+ Aph | 4.6 | 770 | 41 | I |
| 963 T O+ Aph | 7.7 | 546 | 15 | C |
| 932 6 O+ Aph | 5.9 | 200 | 7 | I |
| 978 H O+ Aph | 7.6 | 647 | 13 | C |
| 007 M O+ Aph | 6.7 | 387 | 25 | I |
| 285 K A+ Aph† | 5.7 | 616 | 26 | |
| 635 Q A- Aph† | 5.8 | 1013 | 29 | |
| 008 Q A- Aph | 5.3 | 117 | 4 | C |
| 001 D A- Aph | 6.2 | 725 | 18 | I |
| 862 J A- Aph | 4.3 | 970 | 15 | I |
| 834 R A+ Aph† | 4.8 | 903 | 5 | |
| 291 Z A+ Aph† | 5.1 | 767 | 16 | |
| 412 C A+ Aph | 5.2 | 817 | 68 | I |
| 721 9 A+ Aph | 5.4 | 1043 | 11 | I |
| 091 F A+ Aph | 6.6 | 889 | 24 | I |
| 876 R O+ Aph | 4.5 | 1020 | 57 | C |
| 332 D A+ Aph | 5.9 | 640 | 27 | I |
| 209 N A+ Aph | 5.6 | 1140 | 28 | C |
| 369 C A+ Aph | 5.2 | 355 | 22 | I |
| 599 S A+ Aph | 5.5 | 169 | 16 | C |
| 079 R A+ Aph | 6.8 | 379 | 15 | I |
| 251 Z A+ Aph | 6.8 | 208 | 26 | C |
| 356 M A+ Aph | 8 | 720 | 22 | C |
| 903 0 A+ Aph | 6 | 115 | 19 | C |
| 282 1 A+ Aph | 7.1 | 578 | 33 | I |
| 869 6 A- Aph | 4.4 | 540 | 36 | I |
| 478 D O+ Aph | 6.7 | 793 | 5 | C |

†Platelets transfused together and excluded from analysis C = Compatible I = Incompatible

*Table A29: Pooled platelet donations transfused to patients showing whether they were compatible or incompatible and their CD62P and C1q values, those in red represent values above the normal range.*

| Platelet | MPV | CD62P | C1q | C/I |
|---|---|---|---|---|
| 909 Q Pool A+ | 6.4 | 217 | 11 | C |
| 954 9 Pool O+† | 7.7 | 171 | 5 | |
| 888 Y Pool O+† | 7.1 | 313 | 12 | |
| 915 S Pool O+ | 6.1 | 301 | 3 | C |
| 849 O Pool O- | 7.1 | 79 | 8 | C |
| 812 A Pool A- | 6.8 | 7 | 10 | I |
| 979 8 Pool O+ | 5.6 | 252 | 6 | C |
| 651 S Pool O+ | 6.3 | 205 | 11 | C |
| 762 E Pool A- | 7.4 | 140 | 11 | C |
| 751 P O- Pool | 7.1 | 326 | 16 | C |
| 772 F O+ Pool | 7.4 | 697 | 10 | I |
| 895 U O- Pool | 8.2 | 238 | 5 | C |
| 170 E O- Pool | 6.9 | 477 | 14 | C |
| 667 1 O+ Pool | 6.9 | 643 | 11 | I |
| 306 Z O+ Pool | 7.4 | 226 | 27 | I |
| 258 3 O+ Pool | 5.8 | 386 | 8 | I |
| 630 G O+ Pool | 7.3 | 552 | 5 | I |
| 234 4 O- Pool | 7 | 174 | 13 | I |
| 737 W O+ Pool | 7.3 | 224 | 12 | C |
| 754 E A+ Pool | 7.6 | 396 | 15 | I |
| 451 X O+ Pool | 7 | 155 | 18 | C |
| 647 9 O+ Pool | 6.7 | 247 | 29 | I |
| 294 B O+ Pool | 6.6 | 172 | 13 | I |
| 795 6 O+ Pool | 8.2 | 451 | 20 | I |
| 566 W O- Pool† | 6.2 | 385 | 17 | |
| 782 G A+ Pool† | 7.1 | 260 | 14 | |
| 034 Z A+ Pool† | 7.6 | 327 | 21 | |
| 024 2 O+ Pool† | 8 | 235 | 16 | |
| 244 F O+ Pool† | 7.4 | 154 | 11 | |
| 206 R O+ Pool† | 7.7 | 480 | 20 | |
| 185 5 O+ Pool | 7.8 | 220 | 20 | I |
| 524 * O+ Pool | 7.7 | 139 | 22 | I |
| 819 6 O+ Pool | 7.4 | 120 | 22 | I |
| 814 G O+ Pool | 5.6 | 147 | 16 | I |
| 185 Q O+ Pool† | 6.6 | 533 | 17 | |
| 244 * O+ Pool† | 7.7 | 380 | 20 | |
| 342 W O+ Pool† | 7.1 | 151 | 15 | |
| 859 M A+ Pool | 7.2 | 618 | 15 | C |
| 436 F A+ Pool | 6.5 | 139 | 21 | C |
| 414 B A+ Pool | 7.2 | 243 | 5 | C |
| 490 8 A+ Pool | 7.2 | 640 | 7 | C |
| 895 3 A+ Pool | 5.1 | 285 | 5 | C |
| 087 F A+ Pool | 7.8 | 217 | 11 | I |
| 545 W O- Pool | 6.5 | 182 | 14 | I |
| 461 3 A+ Pool | 7.3 | 125 | 15 | C |

| Platelet | MPV | CD62P | C1q | C/I |
|---|---|---|---|---|
| 705 G A+ Pool | 7.1 | 270 | 10 | I |
| 190 6 A+ Pool | 7.6 | 369 | 13 | I |
| 554 J A+ Pool | 3.1 | 446 | 59 | I |
| 504 2 A+ Pool | 6.8 | 263 | 31 | C |
| 387 1 A+ Pool | 8.5 | 509 | 11 | C |
| 381 D A- Pool | 7.4 | 276 | 12 | C |
| 532 F A+ Pool | 6.2 | 296 | 12 | I |
| 543 9 A- Pool | 6.2 | 470 | 20 | C |
| 742 W A+ Pool | 8.1 | 396 | 18 | C |
| 465 G O+ Pool | 7.5 | 498 | 5 | C |
| 762 J O+ Pool | 6.5 | 246 | 37 | I |
| 635 C A- Pool | 7.8 | 132 | 8 | C |
| 029 O- Pool | 6.6 | 134 | 18 | I |

†Platelets transfused together and excluded from analysis C = Compatible I = Incompatible

*Table A30: Tests for normality using the Kolmogorov-Smirnov test for CD62P, C1q, C3, IgG and C3dg in 51 pooled platelet donations showing that the results do not represent a normal distribution*

| Normality and Lognormality Tests<br>Tabular results | A<br>CD62P | B<br>C1q | C<br>C3 | D<br>IgG | E<br>C3dg | F<br>MPV |
|---|---|---|---|---|---|---|
| **Test for normal distribution** | | | | | | |
| **Kolmogorov-Smirnov test** | | | | | | |
| KS distance | 0.06100 | 0.3218 | 0.1623 | 0.1107 | 0.1182 | 0.1026 |
| P value | >0.1000 | <0.0001 | 0.0018 | >0.1000 | 0.0722 | >0.1000 |
| Passed normality test (alpha=0.05)' | Yes | No | No | Yes | Yes | Yes |
| P value summary | ns | **** | ** | ns | ns | ns |
| **Number of values** | 51 | 51 | 51 | 51 | 51 | 51 |

*Table A31: Tests for normality using the Kolmogorov-Smirnov test for CD62P, C1q, C3, IgG and C3dg in 103 patient platelet samples showing that the results do not represent a normal distribution*

| Normality and Lognormality Tests | A<br>CD62P | B<br>C1q | C<br>C3 | D<br>IgG | E<br>C3dg |
|---|---|---|---|---|---|
| **Test for normal distribution** | | | | | |
| **Kolmogorov-Smirnov test** | | | | | |
| KS distance | 0.1446 | 0.4179 | 0.1725 | 0.09219 | 0.09318 |
| P value | <0.0001 | <0.0001 | <0.0001 | 0.0310 | 0.0279 |
| Passed normality test (alpha=0.05)' | No | No | No | No | No |
| P value summary | **** | **** | **** | * | * |
| **Number of values** | 103 | 103 | 103 | 103 | 103 |

*Table A32: Tests for normality using the Kolmogorov-Smirnov test for CD62P and C1q values in 54 successful transfusions in haematology patients who became refractory to platelet transfusions showing that the results do not represent a normal distribution*

| Normality and Lognormality Tests | A | B |
|---|---|---|
| | P-Selectin | C1q |
| | | |
| **Test for normal distribution** | | |
| **Kolmogorov-Smirnov test** | | |
| KS distance | 0.1711 | 0.1854 |
| P value | 0.0004 | <0.0001 |
| Passed normality test (alpha=0.05)' | No | No |
| P value summary | *** | **** |
| | | |
| **Number of values** | 54 | 54 |

*Table A33: Tests for normality using the Kolmogorov-Smirnov test for CD62P and C1q values in 33 unsuccessful transfusions in haematology patients who became refractory to platelet transfusions showing that the results do not represent a normal distribution*

| Normality and Lognormality Tests | A | B |
|---|---|---|
| | P-Selectin | C1q |
| | | |
| **Test for normal distribution** | | |
| **Kolmogorov-Smirnov test** | | |
| KS distance | 0.1823 | 0.2160 |
| P value | 0.0069 | 0.0004 |
| Passed normality test (alpha=0.05)' | No | No |
| P value summary | ** | *** |
| | | |
| **Number of values** | 33 | 33 |

*Table A34: Search terms used in MEDLINE(R) and Ovid databases 1978 to August 2022.*

| # ▲ | Searches |
|---|---|
| 1 | BLOOD TRANSFUSION.mp. |
| 2 | platelet.mp. |
| 3 | blood platelet.mp. |
| 4 | blood component transfusion.mp. |
| 5 | transfus*.mp. |
| 6 | platelet transfus*.mp. |
| 7 | transfusion.mp. |
| 8 | crossmatching.mp. |
| 9 | platelet specific antibody.mp. |
| 10 | crossmatch.mp. |
| 11 | activation.mp. |
| 12 | platelet activation.mp. |
| 13 | complement.mp. |
| 14 | C3.mp. or Complement C3/ |
| 15 | Complement C1q/ or C1q.mp. |
| 16 | P-selectin.mp. |
| 17 | P-Selectin/ or CD62P.mp. or Platelet Activation/ |
| 18 | activated.mp. |
| 19 | SPRCA.mp. |
| 20 | refractory.mp. |
| 21 | refractoriness.mp. |
| 22 | platelet refractoriness.mp. |
| 23 | compatible.mp. |
| 24 | compatibility.mp. |
| 25 | platelet markers.mp. |
| 26 | apheresis platelets.mp. |
| 27 | pooled platelets.mp. |
| 28 | Platelet Transfusion/ or platelet donation.mp. |
| 29 | increment.mp. |
| 30 | Platelet Transfusion/ or platelet increment.mp. |
| 31 | immune destruction.mp. |
| 32 | flow cytometry.mp. |

# Appendix B

## Higher Specialist Scientific Training module C1 innovation business case

This was in the form of a written assessment and a presentation to Manchester University staff and lay staff as part of the HSST training. The feedback from both is shown here. The contents of the innovation proposal form the basis of the introduction and literature review so are not reproduced here.

# DClinSci C1 Unit Oral Presentation Report

## Section 1: Student

| | |
|---|---|
| **Name of Student:** | Samantha Harle-Stephens |
| **Title of Project:** | Evaluation of the provision of fresh compatible platelets for haematology patients who have become refractory to random platelet transfusions |

## Section 2: Report and Recommendation

| **Please provide a report on the explanation of the work and response to questions.** |
|---|
| Overall a good presentation which demonstrated clarity of explanation of the project at a level suitable to a lay audience whilst demonstrating synthesis of the relevant scientific evidence and the ability to persuade the audience of the merits of the project. Some clarity around the methods being utilised in the study was needed. Also, the importance of platelet additive solution versus plasma has not been considered and this is important in terms of complement presence during storage. The student did use a little bit of jargon such as 'refractoriness' during the presentation however this was clearly explained during the presentation and in the follow-up question and answer session. |
| **Overall Recommendation**<br>**Please tick** |
| Pass |
| **Feedback**<br>*In the event of a fail being awarded, please provide detailed feedback to the student on what is required in order to attain a pass mark.* |
| |

**Examiners:**

Dr Garry McDowell (in the Chair)
Dr Michael Carroll (Reproductive Science)
Dr Nina Dempsey (Haematology and Transfusion Science)
Mr Geoff Lester (NSHCS External Advisor)
Mrs Susan Moore (Lay Representative)

# DClinSci C1 Unit Marking Sheet

## Examiner and Student

| | |
|---|---|
| **Name of Examiner:** | Dr Nina Dempsey-Hibbert |
| **Name of Student:** | Samantha Harle-Stevens |
| **Title of Project:** | Evaluation of the provision of fresh compatible platelets for haematology patients who have become refractory to random platelet transfusions |

## Examiners Report and Recommendation

**Examiners are asked to provide a short report on the literature review and research proposal.**

The literature review demonstrates that the student has fully researched the area but the gaps in the knowledge and how the proposed project could provide new understanding to this area could be clearer.
This literature review will form the basis of the introduction to the doctoral thesis and as such should read as an introduction chapter. There are some key areas that need to be expanded on:

- The area of complement and complement pathways needs further detail, since this is a major part of what is being studied – inclusion of some figures to aid explanation would be welcome.
- CD62P (P-selectin) has been mentioned as something that represents platelet activation, it would also be useful to start with some basic platelet biology – this only needs to be brief but maybe discuss the process of platelet activation and then how CD62P becomes a useful marker of this.

The innovation project contains some vital information that is not present in the literature review (highlighted on the innovation script). As well as reviewing the literature, this introduction needs to set the scene before starting to critique the published studies in this area.
The immunology associated with platelet transfusions in general needs explaining at the beginning in more detail.

There is also a lack of referencing in the first part of the literature review – again this has been highlighted on the document.

The writing style also needs attention in some areas as it can be a little too colloquial.

**Recommendation Please tick**

Pass

# Appendix C

## IRAS and HRA approvals

Copies of the Integrated Research Approval System (IRAS) and Health Research Authority (HRA) approvals for this study are included here.

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Mrs S.J. Reynolds
Trainee Consultant Clinical Scientist in Transfusion
Plymouth NHS Trust
Derriford Hospital level 6
Derriford Road
Plymouth
PL6 8DH

Email: hra.approval@nhs.net
HCRW.approvals@wales.nhs.uk

05 December 2019

Dear Mrs Reynolds

**HRA and Health and Care Research Wales (HCRW) Approval Letter**

| | |
|---|---|
| **Study title:** | **Evaluation of the provision of fresh compatible platelets for haematology patients who have become refractory to random platelet transfusions.** |
| **IRAS project ID:** | **248801** |
| **REC reference:** | **19/LO/1748** |
| **Sponsor** | **University Hospital Plymouth NHS Trust** |

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

**How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?**
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

**How should I work with participating non-NHS organisations?**
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

**What are my notification responsibilities during the study?**

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

**Who should I contact for further information?**
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **248801**. Please quote this on all correspondence.

Yours sincerely,
Helen Penistone
Approvals Specialist

Email: hra.approval@nhs.net
Telephone: 0207 104 8010

*Copy to:* *Dr Christopher Rollinson*

## List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

| *Document* | *Version* | *Date* |
|---|---|---|
| IRAS Application Form [IRAS_Form_18102019] | | 18 October 2019 |
| Letter from sponsor [Sponsorship Letter] | 1 | 02 October 2019 |
| Other [Researcher Responses] | | 16 October 2019 |
| Other [Researcher Responses] | | 16 October 2019 |
| Participant consent form [Participant Consent Form] | 1 | 01 August 2019 |
| Participant information sheet (PIS) [Study Information Sheet] | 1 | 02 October 2019 |
| Participant information sheet (PIS) [Patient Information Sheet] | 2 | 28 November 2019 |
| Research protocol or project proposal [Protocol] | 1 Draft 5 | 01 August 2019 |
| Summary CV for Chief Investigator (CI) [Research CV] | 1 | 02 October 2019 |

| IRAS project ID | 248801 |
|---|---|

### Information to support study set up

The below provides all parties with information to support the arranging and confirming of capacity and capability with participating NHS organisations in England and Wales. This is intended to be an accurate reflection of the study at the time of issue of this letter.

| Types of participating NHS organisation | Expectations related to confirmation of capacity and capability | Agreement to be used | Funding arrangements | Oversight expectations | HR Good Practice Resource Pack expectations |
|---|---|---|---|---|---|
| This is a single site study. | This is a single site study sponsored by the participating NHS organisation. You should work with your sponsor R&D office to make arrangements to set up the study. The sponsor R&D office will confirm to you when the study can start following issue of HRA and HCRW Approval. | This is a single site study sponsored by the participating NHS organisation therefore no agreements are expected. | No external study funding has been sought | A Principal Investigator should be appointed at study sites | The sponsor has confirmed that local staff in participating organisations in England who have a contractual relationship with the organisation will undertake the expected activities. Therefore, no honorary research contracts or letters of access are expected for this study. |

**Other information to aid study set-up and delivery**

| *This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales in study set-up.* |
|---|
| The applicant has indicated that they do not intend to apply for inclusion on the NIHR LCRN Portfolio. |

Health Research Authority

**London - Hampstead Research Ethics Committee**
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

**Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval**

04 December 2019

Mrs S.J. Reynolds
Trainee Consultant Clinical Scientist in Transfusion
Plymouth NHS Trust
Derriford Hospital level 6
Derriford Road
Plymouth
PL6 8DH

Dear Mrs Reynolds,

| | |
|---|---|
| **Study title:** | **Evaluation of the provision of fresh compatible platelets for haematology patients who have become refractory to random platelet transfusions.** |
| **REC reference:** | **19/LO/1748** |
| **IRAS project ID:** | **248801** |

Thank you for your letter of 03 December 2019, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved on behalf of the Chair.

**Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

**Conditions of the favourable opinion**

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Confirmation of Capacity and Capability (in England, Northern Ireland and Wales) or NHS management permission (in Scotland) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

It is a condition of the REC favourable opinion that **all clinical trials are registered** on a publicly accessible database. For this purpose, 'clinical trials' are defined as the first four project categories in IRAS project filter question 2. Registration is a legal requirement for clinical trials of investigational medicinal products (CTIMPs), except for phase I trials in healthy volunteers (these must still register as a condition of the REC favourable opinion).

Registration should take place as early as possible and within six weeks of recruiting the first research participant at the latest. Failure to register is a breach of these approval conditions, unless a deferral has been agreed by or on behalf of the Research Ethics Committee ( see here for more information on requesting a deferral: https://www.hra.nhs.uk/planning-and-improving-research/research-planning/research-registration-research-project-identifiers/

As set out in the UK Policy Framework, research sponsors are responsible for making information about research publicly available before it starts e.g. by registering the research project on a publicly accessible register. Further guidance on registration is available at: https://www.hra.nhs.uk/planning-and-improving-research/research-planning/transparency-responsibilities/

You should notify the REC of the registration details. We routinely audit applications for compliance with these conditions.

**It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).**

**After ethical review: Reporting requirements**

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study, including early termination of the study
- Final report

The latest guidance on these topics can be found at https://www.hra.nhs.uk/approvals-amendments/managing-your-approval/.

**Ethical review of research sites**

The favourable opinion applies to all NHS/HSC sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" above).

**Approved documents**

The documents reviewed and approved by the Committee are:

| *Document* | *Version* | *Date* |
|---|---|---|
| IRAS Application Form [IRAS_Form_18102019] | | 18 October 2019 |
| Letter from sponsor [Sponsorship Letter] | 1 | 02 October 2019 |
| Other [Researcher Responses] | | 16 October 2019 |
| Participant consent form [Participant Consent Form] | 1 | 01 August 2019 |
| Participant information sheet (PIS) [Study Information Sheet] | 1 | 02 October 2019 |
| Participant information sheet (PIS) [Patient Information Sheet] | 2 | 28 November 2019 |
| Participant information sheet (PIS) [Tracked changes] | 2.0 | 28 November 2019 |
| Research protocol or project proposal [Protocol] | 1 Draft 5 | 01 August 2019 |
| Summary CV for Chief Investigator (CI) [Research CV] | 1 | 02 October 2019 |

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**User Feedback**

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

**HRA Learning**

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at: https://www.hra.nhs.uk/planning-and-improving-research/learning/

| 19/LO/1748 | Please quote this number on all correspondence |
|---|---|

With the Committee's best wishes for the success of this project.

Yours sincerely,

**Dr Rahul Chodhari**
**Chair**

Email: NRESCommittee.London-Hampstead@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Dr Christopher Rollinson

# Appendix D

## Patient Information and Consent

This appendix contains a copy of the patient information sheet and consent form as accepted by the IRAS committee.

NHS
University Hospitals Plymouth
NHS Trust

# Participant Information Sheet

## Evaluation of the provision of fresh compatible platelets for haematology patients who have become refractory to random platelet transfusions.

**1. Invitation**

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, and discuss it with others if you wish.

Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

**2. Why have I been chosen?**

You are a haematology patient who requires regular platelet transfusions.
This is due to the effects of your treatment, where these platelet transfusions do not increase your counts enough and consequently you may need to have more transfusions. This is called platelet refractoriness.

**3. What is the purpose of the study?**

Currently platelet transfusions are given using random donor platelets that have not been matched to your blood. The purpose of this study is to see if matching the platelet donations to your blood could raise your platelet count more than if you had random donor platelets which might reduce the number of transfusions

**4. Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form to confirm that you understand what is involved when taking part in this study. If you decide to take part you are free to leave the study at any time and without giving a reason. If you withdraw, unless you object, we will still keep records relating to the treatment given to you, as this is valuable to the study. A decision to withdraw at any time, or a decision not to take part, will not affect the quality of care you receive

**5. What will happen to me if I take part?**

Nothing will happen to you beyond your normal hospital care. Daily blood samples will be taken from you as normal, for analysis. However, if you have a platelet transfusion there will be an additional citrate sample required which will be taken alongside your usual EDTA sample.

**6. What is being tested?**

The platelet transfusions you have will be tested as will your bloods pre and post that transfusion. Testing your blood pre and post transfusion to see what your platelet counts are is normal practice; however, in this study we will be doing additional testing for other proteins whose presence may be the cause of the refractoriness.

**7. What are the possible benefits of taking part?**
We cannot promise the study will help you but the information we get might help improve the treatment of people with platelet refractoriness whilst undergoing treatment for a haematological condition.

**8. What happens when the research study stops?**
You will continue to receive platelets transfusions as part of your normal care. However, the extra tests carried out on your blood samples for the research study for will stop.

**9. What if there is a problem?**
If you do decide to take part in the study there will be no extra samples taken. The investigations will be done on samples already taken as part of your clinical care.

**10. Will my taking part in this study be kept confidential?**
Yes. All the information about your participation in this study will be kept confidential. If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital under the provisions of the 2018 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the trial. You will be allocated a trial number, which will be used as a code to identify you on all trial forms. There is the possibility that one of the documents will contain your hospital number, however this will not appear on the same sheet as any clinical results.

Your records will be available to people authorised to work on the trial but may also need to be made available to people authorised by the Research Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. A copy of your consent form may be sent to the Research Sponsor during the course of the study. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority and Independent Ethics Committee; this is to ensure that the study is carried out to the highest possible scientific standards. All will have a duty of confidentiality to you as a research participant.

If you withdraw consent from further study treatment, your data will remain on file and will be included in the final study analysis.

In line with Trust policy, at the end of the study, your data will be securely archived for a minimum of 5 years. Arrangements for confidential destruction will then be made.

**Privacy Notice for Patients**
University Hospitals Plymouth NHS Trust is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. As mentioned, University Hospitals Plymouth NHS Trust will keep identifiable information about you for a minimum of 5 years after the study has finished in our secure NHS archive.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

If you wish to raise a complaint on how we have handled your personal data, you can contact our Data Protection Officer who will investigate the matter. If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO).

The Trusts Data Protection Officer can be contacted them at:

Data Protection Officer
University Hospitals Plymouth NHS Trust
Information Governance Team
1st Floor Bircham House
William Prance Road
Derriford
Plymouth
PL6 5WR

Tel: 01752 437284
E-mail: informationgovernancepht@nhs.net

For more information about research and about general use of patient information go to https://www.hra.nhs.uk/information-about-patients/

**11. What if new information becomes available?**

If the study is stopped for any reason, you will be told why and your continuing care will be arranged.

**12. What will happen if I don't want to carry on with the study?**

If you decide you do not want to carry on with the study you may withdraw at any time and without giving a reason (although we may ask you for a reason, to help us design better studies for the future, it is up to you whether you are happy to supply a reason or not). If you withdraw, we will still keep records relating to the treatment given to you, as this is valuable to the study and your safety. A decision to withdraw at any time, or a decision not to take part, will not affect the quality of care you receive

**13. Will the study information help with other research projects?**

It is important that good quality research data can be shared with others in order to advance clinical research and to benefit patients in the future. After the end of the study, de-identified information collected during the study may be made available to other researchers under an appropriate data sharing agreement, but it will not be possible to identify you or your family personally from any information shared.

**14. What will happen to the samples I give?**

They are destroyed by incineration after 5 days.

**15. What will happen to the results of this research study?**

The results of the study will be available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication.

Should you wish to see the results, or the publication, please ask your study doctor.

**16. Who is organising and funding this clinical trial?**

This research project is part of a DClinSci which is being funded by Health Education England (HEE).

**17. Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Health Research Authority, the appropriate Research Ethics Committee and the Research Development and Innovation team at Derriford Hospital.

**18. Who should I contact with questions?**

If at any stage you have questions about the study or wish to read the research on which this study is based then please speak to your study doctor or the researcher.

**Doctor:** Dr. Wayne Thomas

**Tel. Number:** 01752 433117

**Researcher:** Samantha Reynolds

**Tel. Number:** 01752 245007

**If you have concerns while on the study**

Whilst it is something we hope will not happen, if you have concerns about any aspect of research please speak to the researchers using the contact details you will have been provided with. Alternatively, you may wish to contact the hospital's Patient Advice and Liaison Service (PALS).

PALS offers support, information and assistance to patients, relatives and visitors and will:

- Provide information about hospital services.
- Offer advice on where to go to get health information.
- Help with problems that you haven't been able to sort out with staff on a ward or in a clinic.
- If you want to make a complaint - advise you how to do so.
- Tell you about independent organisations that can help you with a complaint.
- Listen to your views on how we can improve our services, and pass this on to the appropriate people for action.

PALS can be contacted at:

Patient Advice & Liaison Service
Level 7
Derriford Hospital
Plymouth
PL6 8DH

Tel: 01752 439884
Email: plh-tr.PALS@nhs.net

If you decide you would like to take part then please read and sign and date the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

**Thank you for taking the time to read this information sheet and to consider this study.**

NHS
University Hospitals Plymouth
NHS Trust

# PARTICIPANT CONSENT FORM

## Evaluation of the provision of fresh compatible platelets for haematology patients who have become refractory to random platelet transfusions.

Name of Researcher: S.J.Reynolds

Participant Identification Number for this trial: | | | | | | | | | | |

***Please only initial boxes if you agree with the statements below***

| | | |
|---|---|---|
| 1. | I confirm that I have read the information sheet dated 5th August 2019 (version 1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. | Initial box |
| 2. | I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected | Initial box |
| 3. | I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from [company name], from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. | Initial box |
| 4. | I agree to take part in the above study | Initial box |

| | | |
|---|---|---|
| Name of Participant | Date | Signature |
| Name of Person receiving Consent | Date | Signature |

***When completed: 1 copy for participant; 1 copy in medical notes and original copy for researcher site file.***

# Appendix E

## HSST A Module Results

The thesis forms module C2 of the University of Manchester HSST training.

Module A comprised of five taught units which were examined following the submission of two essays for each unit. The final marks as ratified by the University Board of Examiners are shown in this appendix.


PGDip Leadership & Management in the Healthcare Sciences
Unit marks ratified by Board of Examiners, November 2018

**Trainee name:** Samantha Harle-Stephens
**Student ID:** 10103159
**Award:** PG Credit

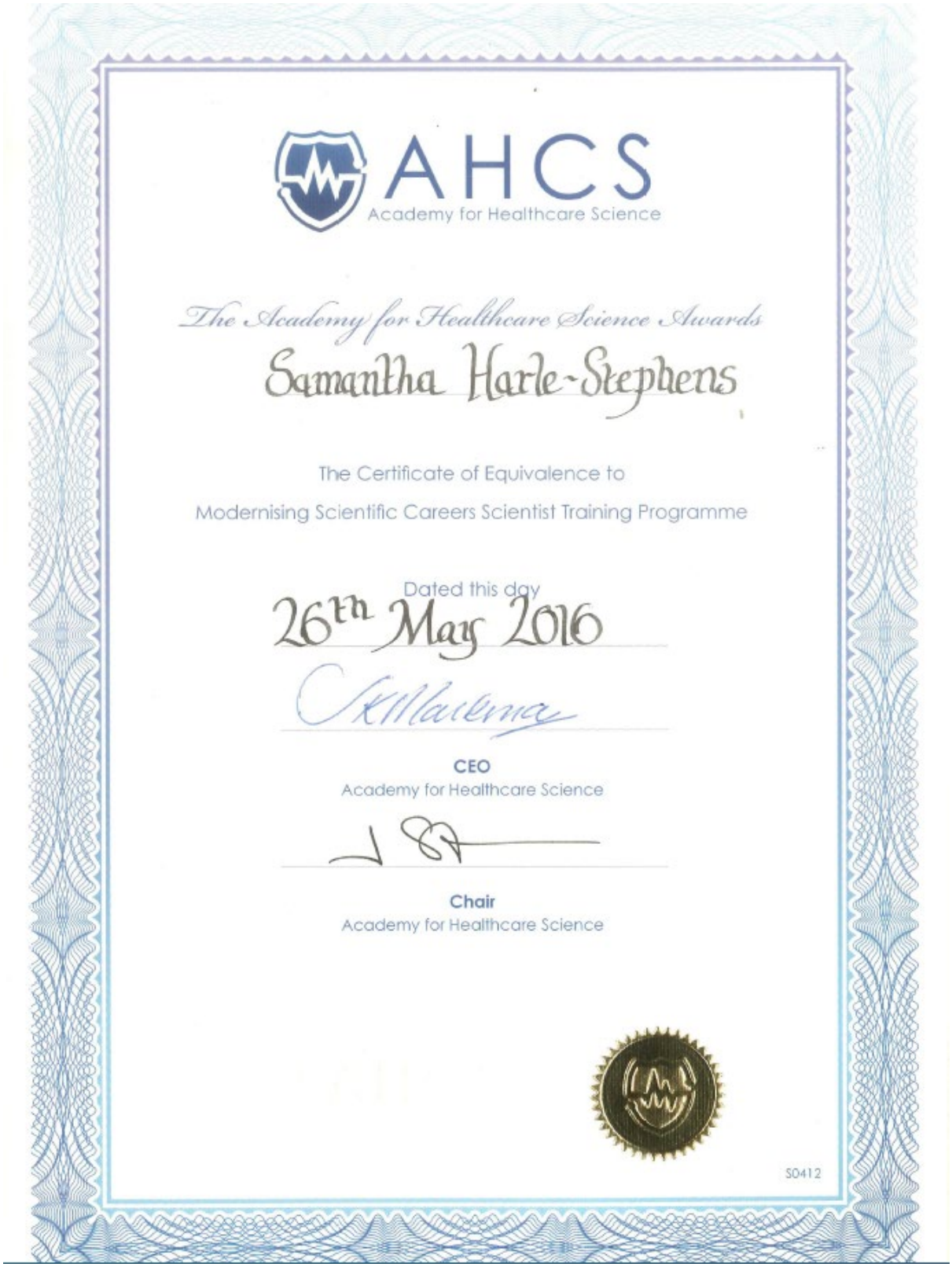
| Unit | Unit Title | Mark | Credits |
|---|---|---|---|
| BMAN73511 | Unit A1<br>Professionalism and Professional Development in the Healthcare Environment | 51%<br>**Pass** | **30** |
| BMAN73522 | Unit A2<br>Theoretical Foundations of Leadership | 66%<br>**Pass** | **20** |
| BMAN73531 | Unit A3<br>Personal and Professional Development to Enhance Performance | 69%<br>**Pass** | **30** |
| BMAN73542 | Unit A4<br>Leadership and Quality Improvement in the Clinical and Scientific Environment | 55%<br>**Pass** | **20** |
| BMAN73550 | Unit A5<br>Research and Innovation in Health and Social Care | 57%<br>**Pass** | **20** |
| | | | **120 / 120** |

# Appendix F

## Qualifications gained during HSST

The completion of this doctorate will form the research component for the Royal College of Pathologists (RCPath) award of fellowship. In addition to the doctorate, applicants must be registered with the Health and Care Professions Council (HCPC) as a clinical scientist and pass the RCPath Part One and Part Two examinations.

The author achieved Equivalence as a Clinical Scientist in 2016 and passed RCPath Part One in 2019. Part Two still has to be achieved. Examination certificates are reproduced in this appendix.



AHCS
Academy for Healthcare Science

*The Academy for Healthcare Science Awards*

Samantha Harle-Stephens

The Certificate of Equivalence to

Modernising Scientific Careers Scientist Training Programme

Dated this day

26th May 2016

CEO
Academy for Healthcare Science

Chair
Academy for Healthcare Science

S0412

# The Royal College of Pathologists


*By these letters make it known that*

## *Samantha Reynolds*

*having undertaken the required training and after having passed the Part One examination in*

## *Transfusion Science*

*has been awarded*

**Diplomateship of**

**The Royal College of Pathologists**

*In witness whereof the Seal of the College and the signatures of the proper Officers have been affixed this thirteenth day of February 2020*

LONDON

*President* *Registrar* *Member of Council*