Optimization of blood component preparation

Processing and donor influence

Bontekoe, I.J.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 1

General Introduction
CHAPTER 1

INTRODUCTION

Blood transfusion as an intervention for treatment of patients is a relative young branch of the medical sciences. The first blood bank in the USA was founded 100 years ago [1], whereas the first blood bank in the Netherlands started soon after the first blood transfusion in 1930. The discovery of the ABO blood group system and citrate as anticoagulant were the first major and basal developments that improved blood transfusion and allowed “banking” of blood.

In the early days of transfusion medicine, whole blood (WB) was administered, but for several reasons, component therapy was introduced in the 1960s and 1970s. As therapeutic components, red cells are administered for maintenance of oxygen transport, platelets to correct low platelet counts, for instance of hemato-oncologic patients, and plasma or coagulation factor concentrate is used to correct deficiency of factor VIII and/or other coagulation factors. Separation of WB into these three main components allowed optimization of component-specific storage conditions and maximization of the storage period and blood usage.

Initially, the blood was collected in glass bottles but these were substituted for plastic containers made from plasticized poly vinyl chloride (PVC). Bag systems with multiple satellite bags were developed to facilitate WB separation technologies, allowing further automation and standardization of the separation process. Another breakthrough was the development of devices for docking PVC tubing, which made it possible to make sterile connections between containers for secondary and tertiary processing of blood components.

Additionally, rather than collecting an entire unit of WB, technology was developed to collect only a part of the blood. With this so-called apheresis technique, one single or multiple components, e.g. plasma and/or platelets and/or red cells, are collected by bedside centrifugation of the blood. Simultaneously, the parts of the blood which are not needed for patient treatment are returned to the donor.

WHOLE BLOOD PROCESSING

In the Netherlands, WB donations of 500 mL are collected from voluntary donors in PVC multiple bag systems filled with an anticoagulant containing citrate, phosphate and dextrose (CPD). Adequate mixing of the blood with this anticoagulating fluid is performed by special collection mixers [2,3]. To improve quality of red cells and plasma clotting factors, the blood is cooled rapidly to room temperature with help of plates filled with butane-1,4-diol plates, a
wax with a melting point of approximately 20°C. By using these plates, it was demonstrated that overnight hold for a maximum of 24 h results in acceptable quality of components, as well as giving units the same temperature history [4,5]. In most European countries, WB is held at room temperature and processed within 24 h, while in the USA, WB held at room temperature, has to be processed within 8 hours when all components are used.

The first and basic step of WB separation is centrifugation. In the Netherlands, a hard spin (4,790 x g) is applied followed by separation with a semi-automated press into three components: red cells, plasma and buffy coat (BC). Harvesting of the BC-layer was introduced in the 1980s to remove a substantial part of the leukocytes and platelets, thereby preventing formation of microaggregates and improving red cell concentrates [6]. Subsequently, BCs were used to isolate platelets, which were less activated compared to the ‘platelet-rich plasma’ method [7-9]. Fine-tuning and improvement of centrifugation and the semi-automated presses has led to the production of highly standardized blood components. Alternatively, if no platelets need to be isolated, WB collections can be first filtered to remove leukocytes and platelets, and then separated into red cells and plasma. To minimize operator interventions, fully automated devices for WB separation have been developed after the year 2000, which combine centrifugation and expression of components to satellite bags in a one-step process [10].

An important component for blood product quality control is temperature control during all process and logistic steps, ranging from collection to storage and delivery. As indicated above, butane-1,4-diol cooling plates gave good quality of all blood components, and later studies revealed that the use of cooling plates for overnight hold contributed substantially to better in vitro quality of red cell concentrates (RCCs), without beneficial effects on factor VIII levels in plasma, on BC cellular composition or platelet quality [11,12]. The temperature during WB processing is kept further under control by climate control of the processing department and the use of temperature-controlled centrifuges. As a rule, centrifugation temperature for WB to be used for platelet production is kept between 20-25°C [13].

**RED CELL CONCENTRATES**

Red cells are highly specialized cells without a nucleus, mitochondria or other organelles. Red cells contain the oxygen-binding protein hemoglobin, to achieve transport of oxygen from the lungs to other tissues. Immediately after separation of WB, the red cells are suspended in a synthetic medium, called an additive solution (AS), containing basic ingredients the cells
need to survive outside the body and to guarantee a shelf life of at least 35 days. In Europe, the most common additive solution is saline-adenine-glucose-mannitol (SAGM) while in the USA AS-1 and AS-3 are most commonly used, which also contains saline, adenine and glucose, and mannitol (AS-1) or citrate and phosphate instead of mannitol (AS-3) [14]. After mixing with the additive solution, the red cells are filtered to remove leukocytes and platelets that are trapped during centrifugation in the red cell compartment of the original WB bag. This filtration of RCCs was introduced as a purification step but also improved storage conditions [15]. Depending on the additive solution used as well as local or national regulations, RCCs are stored at 2-6°C for 21-49 days [16].

Soon after filtration, the RCCs are refrigerated to slow down metabolism and to prevent bacterial outgrowth. An immediate effect of the low temperature is that the Na-K pump stops, which causes leakage of K⁺ into the medium and a strong rise of free K⁺ during storage of RCCs. The pump is driven by the enzyme Na/K-ATPase, which depends on adenosine triphosphate (ATP), mainly generated by glycolysis (Figure 1). To maintain glycolysis and ATP levels, many additive solutions contain glucose and adenine, while mannitol is added to delay hemolysis. It has been shown in transfusion experiments that ATP levels should be maintained above 2.7 µmol/g Hb [5,17,18]. In the circulation, red cells also need 2,3-diphosphoglycerate (2,3-DPG) to allow proper off-loading of oxygen by hemoglobin. It is synthesized in the Rapoport-Luebering shunt of glycolysis, a reaction that is pH dependent. During storage, the 2,3-DPG levels decrease gradually to zero within approximately 14 days, a process that starts immediately after collection. This decline is sensitive to pH, temperature history and composition of the additive solution. The end-product of (anaerobic) glycolysis, lactate, accumulates during storage and is the main cause for further acidification of the red cell milieu with an inhibition of 2,3-DPG synthesis as side effect. This side effect has never been considered as a serious problem for transfusion due to the regeneration of 2,3-DPG inside the recipient of a red cell transfusion [19] but newly developed additive solutions prevent the 2,3-DPG decline and make RCCs a transfusion product with much higher consistency [20].

In stored red cells, also oxygen radicals and peroxides are generated, so called reactive oxygen species (ROS), which lead to oxidation of hemoglobin to methemoglobin and gives negative effects on the membrane network and on the enzymes of the glycolysis. Damage by free radicals is repaired with the help of reduced glutathione (GSH), and cells maintain a high ratio of reduced to oxidized glutathione with the help of the pentose phosphate pathway (Figure 1). Metabolic markers to characterize RCCs are pH, glucose consumption and lactate production, ATP and 2,3-DPG content. Markers for cell-integrity are hemolysis,
Figure 1: Metabolic pathways of red cells (in red) and platelets (in red and black).

Glycogenesis / glycogenolysis
- glycogen $\rightarrow$ glucose 1-phosphate
- glycerol $\rightarrow$ glyceraldehyde 1-phosphate

Rapoport-Luebering pathway
- 2,3-diphosphoglycerate $\rightarrow$ 1,2-diphosphoglycerate (x2)
- 3-phosphoglycerate (x2)
- 2-phosphoglycerate (x2)
- phosphoenolpyruvate (x2)
- pyruvate (x2)
- lactate (x2)

Glycolysis
- glucose $\rightarrow$ glucose 6-phosphate $\rightarrow$ fructose 6-phosphate $\rightarrow$ fructose 1,6-biphosphate
- glyceraldehyde 3-phosphate

Pentose phosphate pathway
- NADP$^+$ $\rightarrow$ NADPH
- 2 GSH $\rightarrow$ GS-SG$^*$
- ribose 5-phosphate

Amino acids
- fatty acids
- acetate
- acetyl-CoA

Tricarboxylic acid cycle
- oxaloacetate
- malate $\rightarrow$ fumarate $\rightarrow$ succinate

* GSH: Glutathion, reduced, GS-SG: Glutathion, oxidized
potassium leakage, osmotic fragility, deformability, morphology, mean corpuscular volume and microvesicles.

With the aim to reduce donor exposure, neonates and young children are treated with aliquoted RCCs, so-called pedipacks [21]. For treatment of hemolytic disease of the fetus and newborn (HDFN), which is anemia caused by allo-antibodies of the mother, special red cell products were developed. A RCC for intrauterine transfusion is prepared by removal of SAGM by high speed centrifugation to remove adenine and mannitol, substances that have possible renal toxicity in fetuses and pre-term infants. The RCC is selected after showing compatibility with the maternal antibodies. After removal of the supernatant, the hematocrit is adjusted to 80-85% by adding 0.9% saline. Because the immune system of a fetus is not yet developed, RCCs for intrauterine transfusion are always gamma irradiated to prevent DNA replication of the remaining white blood cells, to prevent transfusion-associated graft-versus-host disease (GvHD). When a baby is diagnosed with HDFN, its blood can be exchanged with reconstituted whole blood, where the SAGM of a RCC is replaced with AB plasma to a hematocrit level of 50%.

PLATELET CONCENTRATES

To prepare a platelet product suitable for transfusion, BCs undergo a secondary process. Four to six BCs, each containing about 90% of platelets originally present in the WB, 70% of white cells and some red cells suspended in plasma, are pooled in a pooling bag system, together with a unit of plasma or platelet additive solution (PAS). A soft spin (~700-1300 x g, dependent on the storage medium) is applied for sedimentation of red cells and leukocytes, leaving the platelets suspended in the supernatant. This suspension is subsequently expressed to a storage container through a filter, resulting in a leukoreduced platelet concentrate (PC). In the Netherlands, universal leukocyte reduction of PC was introduced in 2002. Depending on the quality of the storage medium as well as local regulations, PCs are stored at 20-24°C for 4-7 days. In the Netherlands, PCs were prepared from 5 BC and plasma with a storage time of 7 days, or PAS-B with storage time of 5 days. PAS-B is a salt-containing medium with only acetate as a substrate for the platelets and citrate for anticoagulation. To improve quality and to increase storage time of PC in PAS also to 7 days, PAS-B was substituted for PAS-C in 2015. PAS-C contains phosphate for pH buffering. In 2018, the dual inventory of platelets in both plasma and PAS-C was replaced for an inventory only with platelets in PAS-E, a next generation additive solution containing - besides citrate, acetate
and phosphate - also magnesium and potassium. The combined effect of these electrolytes in PCs results in lower lactate production and lower activation of platelets [22,23]. Use of PAS for BC-derived platelet concentrates results in a more standardized product, fewer adverse reactions [24-26] and increased availability of plasma for therapeutic use.

PCs are also collected by apheresis techniques in plasma or PAS, resulting in single-donor concentrates, having the advantage of minimal donor exposure. In the Netherlands, less than 10% of the platelet products are collected by apheresis in plasma, mainly for special patient groups such as neonates and patients who need human leukocyte antigen (HLA) or human platelet antigen (HPA) matched platelets, because of refractoriness to regular PCs.

Platelets are cell fragments without a nucleus, but, unlike red cells, have different granules and also contain mitochondria. As a result, they have many metabolic pathways and they can use many different substrates as fuel (Figure 1). With respect to platelet storage, the rate of glycolysis has always been considered as important because glucose is metabolized into pyruvate which may be further metabolized into lactate, resulting in acidification of the PC with subsequent loss of quality. Glycolysis is performed in the cytosol, and, as recently discovered, also in the α-granules [27]. Uptake of glucose by platelets is mainly facilitated by the glucose transporter GLUT-3, that has a Km for glucose of ~1.5 mmol/L. This means that under physiological conditions (~5 mmol/L) and conditions in a PC with glucose concentrations of 5-20 mmol/L for PC in PAS or CPD-plasma, it functions nearby saturation. Platelets contain also a minimal amount of GLUT1 [27-29]. In the presence of glucose, increased lactate production was observed in washed platelets after addition of insulin [30], but the role of insulin for uptake of glucose by stored platelets in PAS or plasma has, to our best knowledge, not been examined. However, platelets are known to have insulin receptors, insulin receptor substrate-1 (IRS1) and other elements of the insulin signaling pathway [31].

Platelets can store glucose as glycogen in their glycogen granules, and upon activation they will also use it as fuel [27], so they contain all enzymes of the glycogenesis and glycogenolysis pathways. Pyruvate and many other substrates like fatty acids, glycerol from lipids, amino acids like glutamate, citrate from anticoagulant and/or PAS and acetate from PAS are all metabolized in pathways, taking place in the mitochondria (Figure 1) [32,33]. Like red cells, platelets shunt glucose-6-phosphate into oxidation to pentose phosphates, a pathway used by cells to maintain a high ratio of reduced to oxidized glutathione, to prevent free radical damage. ATP and other nucleotides, involved in these catabolic pathways, are part of what is called the metabolic pool. Platelets also have a storage pool of ATP and ADP in their dense granules, which is released when they become activated.
Upon activation of platelets, induced either by biochemical agonists, shear stress due to processing or contact with plastic surfaces, they will secrete (a part of) the α-granules, dense granules and lysosomes, and also fusion of granule membranes with the plasma membrane will take place. As a consequence of α-granule release, the endothelial adhesion protein CD62P will be expressed on the platelet surface. In addition, many other adhesive glycoproteins such as GPIIb/IIIa, coagulation factors and protease inhibitors that promote adhesion, aggregation and participation of the platelet in hemostasis, will be delivered into its surroundings. The hemostatic function of platelets is supported by release of lysosomes, resulting in expression of CD63 on the plasma membrane [34] and release of dense granules, which are stores of ADP, ATP, Ca\(^{2+}\) and Mg\(^{2+}\). Due to influx of Ca\(^{2+}\), there is also loss of membrane asymmetry, and phosphatidylserine is expressed on the platelet surface [35]. Metabolic consequences of platelet activation are increased glucose uptake (due to GLUT3 upregulation), glycolysis and lactate production, utilization of glycogen and depletion of Ca\(^{2+}\) and ATP.

Platelet quality deteriorates during blood bank storage, and to slow down this process, many technical measures have been implemented. One such measure was the introduction of pre-storage leukocyte-depletion of PCs in 2002, which reduced cytokine levels, transfusion reactions and transmission of cell-bound viruses like cytomegalovirus. The discovery that PCs are best stored at room temperature has led to the development of special storage containers and equipment. Storage containers were optimized for material and surface and are nowadays mainly made from PVC, plasticized with e.g. butyryl-trihexyl citrate (BTHC), a highly gas-permeable material. To facilitate the influx of oxygen and efflux of carbon dioxide, PCs are also stored with continuous gentle agitation [36,37]. Storage at 22°C may, however, lead to bacterial growth if the blood was contaminated by for instance skin flora, and recipients of PCs are thus at risk for bacterial infection. To reduce the risk of sepsis for the recipients to a minimum, many countries limit shelf life of PC to 5 days. Otherwise, when 7-day storage is wanted, as in the Netherlands, culturing PC samples for bacterial screening is mandatory [38].
PLASMA

After expression of plasma to a satellite bag, the plasmas are quickly frozen using liquid nitrogen or blast-freezers and subsequently stored below -25°C for not more than 2 years. This so-called recovered plasma is used for preparation of clotting factor concentrates like factor VIII or IX or for direct therapeutic purposes [39]. In the Netherlands, plasma for therapeutic purposes is collected by apheresis, and, after 2013, exposed to a solvent-detergent treatment to reduce pathogens [40].

PATHOGEN INACTIVATION

Some measures to guarantee the microbial safety of plasma and PCs were already mentioned. Together with other measures like the donor questionnaire, screening for bacteria in platelet concentrates and screening of every donation for some highly pathogenic blood transmissible viruses and bacteria like hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and Treponema pallidum, these measures make transfusion of blood products nowadays a safe medical treatment. However, different approaches, not based on prevention of microbial contamination but based on treatment of the blood (components) to inactivate possible contamination with (unknown) pathogens, have come on the market or are under development. These pathogen inactivation techniques inactivate a broad spectrum of viruses, parasites and bacteria by inactivation of their DNA and RNA. The three methods applied for inactivation of PCs use UV light and in two cases also an active component is added (Amotosalen-HCl respectively riboflavin). Pathogen inactivation also affects platelet quality and worsens the storage lesion [41,42], so the clinical efficacy is somewhat reduced [43,44].

QUALITY OF BLOOD COMPONENTS

Although quality of blood products has reached high standards and transfusion is a life-saving therapy, transfusion of red cells or platelets has not only benefits but may also contribute to morbidity and mortality [45]. In the past decades, measures have been taken by hospitals to increase transfusion efficacy and reduce the use of blood, for instance by implementation of blood-saving surgical techniques, the use of cell saving devices and lowering transfusion triggers. Considering the storage lesion of red cells and platelets and their potential harmfulness, theoretically a fresh product would be better than a stored product.
It is known that depending on storage time, up to 25% of the red cells is lost from the circulation within the first 24 h after transfusion [46]. However, several clinical studies, performed to investigate transfusion of fresh versus stored red blood cells, showed that there is no reason for the preferred administration of fresh units, but the safety of red cell units stored for more than 35 days has not been established [47-51]. In case of platelet transfusions, count increments after transfusion of 2-day old PCs were significantly higher than after transfusion of 5- or 7-day old PCs. Seven-day storage had no effect on transfusion outcome, compared to 5-day storage [52], or the effects observed were considered as clinically irrelevant [53]. From that point of view and, after reaching such a high standard of blood component quality and logistic processes, current research focus in transfusion medicine has shifted to better storage performance rather than an increase in storage time. So, quality of blood components is still a challenge for blood centers and subject of ongoing research. In addition, consistency (or a constant product quality) is a relatively new requirement, because a blood component destined for transfusion will be considered more and more as a drug with similar product requirements as drugs produced by the pharmaceutical industry. Hence, further research in product consistency and improvement of cellular blood products is warranted.

**AIM OF THIS THESIS**

Methods and techniques to guarantee a high level and improving blood component quality are for instance donor selection, gentle processing, visual inspection of components and temperature control during the whole blood supply chain. Quality of blood transfusion is also improved when recipients get exposed to blood components from a minimal number of donors. *The main purpose of the studies described in this thesis was to improve and to optimize the quality of red cell and platelet products destined for transfusion.*

Several steps in the current process for preparation of blood components from whole blood, were studied in detail, and are described in *part I*. The performance of a new generation semi-automated device for the separation of centrifuged whole blood or pooled buffy coats was tested (*Chapter 2*), with the aim to get more standardized blood products. A substantial part of the whole blood process consists of logistic steps and holding periods, where temperature control is an important tool to maintain quality. Two such holding periods were critically examined. First the (pre-centrifugation) holding time of whole blood during overnight storage was studied with the aim to assess the impact of temperature deviations
General Introduction

beyond the current limits (Chapter 3). Second, the effect of the post-filtration handling of red cell concentrates in SAGM, that is holding at room temperature and cooling to 4°C, was studied (Chapter 4). Finally, with the aim to reduce donor exposure for fetuses, a new preparation method for red cells for intrauterine transfusions was developed (Chapter 5).

Donor influence on the quality of platelet products is the theme of part II. Thromboelastography is a technique to determine the ability of blood to coagulate. Besides being used for patient samples, we applied it to distinguish donor effects on the storage of platelet concentrates. The introduction of thromboelastography as a functional clotting test for blood and blood components has been reviewed (Chapter 6). Introduction in our laboratory has led to the development of a functional thromboelastographic assay for platelet concentrates (Chapter 7). This study demonstrated that thromboelastography was in agreement with other (functional) tests indicating that in some cases the storage performance of platelet

Figure 2: Current processing (2018) in the Netherlands of whole blood for preparation of blood components and of platelet apheresis products. Investigated methods and conditions used in this thesis are in red.
concentrates was related to the donor. With the aim to prospectively show that storage performance of platelets was indeed related to donor and donor health, a second, confirmatory study was performed with platelet concentrates obtained from older donors (Chapter 8). It was found that metabolic syndrome and Type 2 diabetes are likely causes for poor platelet storage properties. Further exploration of this finding was done by investigating platelet concentrates obtained from young donors and older donors with and without Type 2 diabetes (Chapter 9).
REFERENCES

5. Van der Meer PF, Pietersz RN. Overnight storage of whole blood: a comparison of two designs of butane-1,4-diol cooling plates. Transfusion 2007;47:2038-2043.
12. Van der Meer PF, de Korte D. Active cooling of whole blood to room temperature improves blood component quality. Transfusion 2011;51:357-62.
20. de Korte D, Kleine M, Korsten HG, Verhoeven AJ. Prolonged maintenance of 2,3-diphosphoglycerate acid and adenosine triphosphate in red blood cells during storage. Transfusion 2008;48:1081-1089.


51. Remy KE, Sun J, Wang D, et al. Transfusion of recently donated (fresh) red blood cells (RBCs) does not improve survival in comparison with current practice, while safety of the oldest stored units is yet to be established: a meta-analysis. Vox Sang 2016;111:43-54.