Optimising quality of platelet transfusions
Kostelijk, E.H.

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: http://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
GENERAL INTRODUCTION

Together with the coagulation system, platelets constitute a major defence of the organism against blood loss. Among circulating blood cells, platelets are the smallest. They have a diameter of about 4 μm, are about 1 μm thick and their volume is about 7 femtoliter [1]. Main platelet constituents are granules, containing coagulation factors and other solutes such as calcium, ADP, ATP, β-thromboglobulin, platelet factor 4, fibrinogen, platelet-derived growth factor, factor V, thrombospondin, fibronectin, albumin histidine-rich protein, high-molecular weight kininogen (HMWK), alpha-antitrypsin etc. On the outer cell membrane, platelets express membrane glycoproteins that function as receptors for agonists. These receptors can induce platelet activation (granule secretion) and play a role in platelet aggregation, binding to leukocytes and adhesion to surfaces such as a damaged vascular wall and artificial surfaces. The processes of adhesion, aggregation and release of platelet constituents are essential for the formation of the haemostatic plug.

In healthy individuals, platelet numbers vary between 150 and \(300 \times 10^9\) per litre of blood. Due to a variety of diseases, serious blood loss or treatment with cytostatic agents, platelet numbers may drop below a critical level, which poses serious risks, including death. Because platelets are essential for the prevention of spontaneous or posttraumatic bleeding, there may be a need to transfuse platelets when serious shortage exists or is pending. Besides determination of the platelet count, the bleeding time is one of the parameters to measure the effectiveness of platelet transfusions. The bleeding time is prolonged when the platelet count has dropped below \(50 \times 10^9\) per litre or when platelet function is disturbed (such as after administration of certain drugs).

Platelet transfusions are indicated in patients with a serious shortage of platelets combined with an increased risk of bleeding. A less restrictive transfusion policy will enhance the risk of alloimmunisation, which complicates further therapy. Clinical studies suggest that platelet transfusions are only needed in the absence of overt bleeding when the number of platelets is \(10 \times 10^9\) platelets per litre of blood or less [2-4]. For this purpose, a dose of \(240-300 \times 10^9\) platelets per transfusion is administered. These platelets are collected by apheresis or from 4-6 whole blood donations and pooled after isolation.

In The Netherlands, about 225,000 out of a total number of 713,000 donor units of whole blood collected in 1998 were used for preparation and transfusion of platelet concentrates (PCs). Besides PC derived from whole blood units, about 2,600 PCs were prepared via apheresis and used for transfusion. During the last decade, the number of units used for PC preparation increased from 118,000 in 1986 to 233,000 in 1996; thereafter, the number more or less stabilised. The number of apheresis PC increased from 1,600 in 1987 to 2,600 units in 1995, with a minimum of 1,300 units in 1989 (Annual reports 1995 and 1998 of Sanquin Blood Supply Foundation, The Netherlands).
1.1 Origin of platelets

Platelets are anucleate fragments of megakaryocytes. Megakaryocytes are derived from pluripotent hematopoietic stem cells in the bone marrow, through a process that is regulated by cytokines and growth factors. The growth of the most primitive human stem cells (so-called high-proliferative-potential colony-forming-unit megakaryocytes) is regulated by several not yet precisely characterised factors. In murine models, however, comparable cells differentiate through IL-1, IL-3, IL-6, IL-11 into burst-forming-unit megakaryocytes, which express CD34 (stem cell marker) and c-kit (stem cell factor receptor) molecules on their surface [5;6]. These cells are capable of producing numerous colony-forming unit-megakaryocytes, which differentiate in promegakaryoblasts and subsequently into megakaryoblasts. Upon maturation, megakaryoblasts acquire specific platelet membrane integrins such as glycoprotein Ib (Gplb) and GpIIb/IIIa, after which mitosis is not possible anymore. These cells have the unique ability to carry out endoreduplication that results in mature megakaryocytes with lobulated nuclei and abundant cytoplasm, producing hyperploid cells up to 128N. After invagination of the megakaryocyte cell membrane (demarcation membrane containing Gplb and GpIIb/IIIa), platelets are formed by a process of shedding. It is thought that this occurs via transendothelial pseudopods that arise from the megakaryocyte and traverse the vascular endothelium, allowing megakaryocyte cytoplasmic fragments access to the circulating blood. In healthy individuals about 200 x 10⁹ platelets per day are formed through this process. About 30% of these platelets are initially stored in the spleen, where they are exchanged with circulating platelets. After a life span of 9 to 14 days, circulating platelets are broken down by the reticulo-endothelial system [5].

1.2 Blood coagulation, function of platelets

For haemostatic plug formation, platelets form aggregates after adhesion, activation and secretion of their granule contents, while the process of coagulation produces fibrin deposits through a series of enzymatic steps that culminate in thrombin and fibrin generation [7;8]. Haemostasis is a complex system of activating and inhibiting processes in which blood vessels, platelets, coagulation factors and coagulation inhibitors and fibrinolysis play a role.

The principal function of platelets is the formation of an aggregate (thrombus) or haemostatic plug and to provide substances, which stimulate the release reaction and aggregation of platelets and activation of the coagulation system. The process of platelet activation starts with adhesion of platelets to collagen fibres in the vessel wall upon vascular injury [9;10]. Within a few seconds foreign surfaces may also induce platelet adhesion and activation [11]. Collagen fibres activate both platelets and clotting factor XII via so-called contact activation [12]. Activated factor XIIa initiates the intrinsic coagulation cascade [11], a series
of closely linked proteolytic reactions during which clotting factors are converted from an inactive into an activated state, finally leading to fibrin formation (fig. 1.1).

### Intrinsic pathway

![Diagram of the intrinsic and extrinsic coagulation pathway]

**Intrinsic pathway**

**Extrinsic pathway**

Fibrin fibres function as a net to capture platelets, leukocytes and red cells, and once a thrombus is formed, clot retraction expels water from the plug. The second coagulation pathway, called extrinsic, starts when tissue thromboplastin is set free after injury and forms a complex with factor VII that, in turn, becomes activated. Activated factor VII initiates activation of factor X (after which both coagulation pathways follow a common route) which starts the conversion of prothrombin into thrombin. This leads to conversion of fibrinogen in fibrin monomers that after polymerisation form fibrin fibres mainly formed outside the blood vessel [13]. For further details see Hutton *et al.* [5].

Besides collagen fibres, platelets are also activated by agonists such as von Willebrand factor (vWF), which is synthesised by endothelial cells and megakaryocytes. vWF is stored in endothelial secretory granules, called Weibel-
Palade bodies, and in alpha granules of platelets. Thrombin, histamine, and other mediators of thrombosis or inflammation can cause acute release of Weibel-Palade bodies through an increase in intracellular free calcium and a cascade of intracellular reactions [14-16]. Activation of platelets involves a number of trans-membrane signalling events, oxygen consumption and cytoplasmic reorganisation/pseudopod formation, accompanied by granule secretion. Agonists like collagen, thrombin, ADP, phorbol esters and epinephrine induce platelet alpha-granule release. Following the release of alpha granules and Weibel-Palade bodies, free vWF can bind to membrane glycoproteins on collagen and platelet membranes [14;16-19].

Platelet adhesion to sub-endothelium or perivascular connective tissue upon vessel wall damage requires a high shear rate and binding between GpIb and vWF [7;20]. It has been hypothesised that high shear rates (600-3000 s^{-1}) alter the conformation of GpIb and/or vWF, whereas at static conditions, GpIb and vWF have no affinity for each other. GpIb is a disulphide-bonded heterodimer composed of an alpha (143 kDa) and beta (22 kDa) chain. This dimer is non-covalently associated with a separate glycoprotein IX. This complex (GpIb-IX) is the surface receptor for platelet adhesion (fig. 1.2). vWF forms a bridge between type VI-collagen expressed on the sub-endothelial surface and GpIb in the complex. Vascular endothelial cells secrete vWF to both the lumen and the sub-endothelial surface.

![Platelet membrane](image)

**Fig. 1.2** - GpIb, a disulphide-bonded heterodimer composed of an alpha and beta chain.

Adhesion of platelets is not related to vWF and GpIb interaction and occurs at shear rates less than 600 s^{-1}. Activated as well as non-activated platelets are both involved in adhesion. Molecules on the platelet membrane belonging to the integrin family of receptors mediate platelet adhesion [5].
To activate the coagulation system and promote clot formation, platelets contain the following structures:

1. A plasma membrane covered with adsorbed proteins such as von Willebrand-factor (GpIb-IX), [5];
2. A cytoskeleton existing of microtubuli and different proteins that maintains the discoid shape of the platelet [5];
3. Secretion granules which can be divided in dense granules, alpha granules and lysosomal granules [5;21];
4. A tubular system that can be divided into surface connecting tubules that function as excretory duct, and a dense tubular system where prostaglandin synthesis and calcium deposit are situated [5];
5. Mitochondria in which the oxygen-dependent energy supply is located [5];
6. Glycoproteins (membrane receptors) on the outer membrane that can bind to white cells and other platelets after activation [22; 23].

For more detailed information about the regulation of haemostasis and the role of platelets, see Hutton et al. [5].

1.3 Platelet abnormalities

Platelet abnormalities include abnormal platelet number as well as decreased platelet function. In transfusion practice, severe thrombocytopenias are treated with platelet transfusions. Thrombocytopenias, which indicate disorders due to platelet function aberrations, are usually not treated by platelet transfusions except for cases in which an acute bleeding crisis occurs. Thrombocytopenias will not be discussed in this introduction (for further reading see Bellucci-Sessa et al. [24].)

A severe drop in the number of platelets (thrombocytopenia) may occur as a result of decreased platelet production due to bone-marrow failure in diseases such as leukaemia, myelodysplastic syndrome and by certain drugs, or infiltration of bone marrow by solid tumour metastases. Furthermore, thrombocytopenia is a frequent side effect of cancer therapy due to toxic effects on bone-marrow cells.

A second mechanism for the development of thrombocytopenia is excessive platelet consumption, which occurs in patients with various auto-immune disorders such as idiopathic thrombocytopenic purpura, splenomegalta, sepsis and diffuse intravascular coagulation (for detailed review see George et al. [25]).

It is now possible to discriminate between a lack of platelets due to increased platelet destruction versus decreased production, by measuring the glycocalicin level and the thrombopoietin (TPO) level in plasma or serum [26-29]. Glycocalicin, a proteolytic fragment of GpIb on the platelet membrane containing the vWF-binding site, is set free in the supernatant after platelet activation or destruction. TPO is a growth factor of megakaryocytes that stimulates maturation and proliferation of these cells, thus influencing the platelet number. In primary and
secondary idiopathic autoimmune thrombocytopenia (AITP), TPO levels are within the normal range or in slightly increased some cases, whereas glycoprotein levels are normal. In contrast, patients with amegakaryocytic thrombocytopenia have markedly elevated TPO levels and significantly decreased glycoprotein levels. In thrombocytopenia accompanying various disorders, elevated TPO levels are only found when platelet production is depressed. The mean level of glycoprotein in these patients is decreased compared to that in both controls and patients with AITP, but not as low as in amegakaryocytic thrombocytopenia.

1.4 Indications for platelet transfusions

When the number of platelets drops below a critical level, a risk of bleeding occurs. Therapeutic platelet transfusions are given when there is overt bleeding and the platelet count is lower than 50 x 10⁹ per litre.

In the absence of other conditions that may increase the bleeding tendency, such as trauma or surgery, a threshold of 10-20 x 10⁹ platelets per litre is set as a requirement for prophylactic platelet transfusions. Higher threshold levels are applied when other factors leading to increased bleeding are present such as sepsis and concurrent use of drugs (e.g. antibiotics) that affect platelet function.

Prophylactic transfusions are needed to prevent bleeding mainly in patients with haematological malignancies with bone marrow failure. Based on the expected post transfusion recovery and life span of transfused platelets, a dose of about 50 x 10⁹ platelets/kg body weight has been recommended.

1.5 Preparation of platelet concentrates

Platelets are transfused as concentrates prepared from whole-blood units or collected by apheresis. When prepared from whole blood, two methods are used: the platelet-rich plasma (PRP) method, mainly used in North America, and the buffy-coat (BC) method which is mainly used in Europe. When apheresis is used, one platelet concentrate is made from one donation. In this case, platelets and plasma are removed from donor blood while red cells are returned to the donor during the apheresis process. This is achieved by simultaneous centrifugation of the collected blood during a donation, allowing the immediate return of the red cells to the donor and collecting the separated platelets and plasma from the blood. However, the latter method which provides platelet concentrates with low leukocyte contamination is rather time-consuming (60-90 minutes) compared to the collection of whole blood units as starting material (about 10 minutes).
1.5.1 Platelet concentrates prepared by the platelet-rich plasma (PRP) method

Applying the PRP method, PCs are prepared from whole blood units (which can be stored overnight at 20°C). With a soft-spin centrifugation step, the blood is separated into red cells, leukocytes, and a supernatant fraction, platelet-rich plasma. PRP is transferred to a second bag and then centrifuged at high speed to pellet the platelets. The surplus of plasma is removed and the pelleted platelets are resuspended in 60 ml of residual plasma (fig. 1.3). The process of pelleting platelets against the plastic container may cause platelet activation [32].

1.5.2 BC-derived platelet concentrates

After (overnight) storage at 20°C, whole blood is centrifuged and separated into plasma, BC and red cells [30]. The BC, which contains >90% of platelets, >75% of leukocytes and only 5% of red cells [31], is used as the source of platelets. After a second centrifugation, platelet-rich supernatant is separated from pelleted white cells and red cells and transferred into a special container or an empty SAGM container (fig. 1.3). In this way, PCs of about 60 ml with an average concentration of about $1 \times 10^9$ platelets per ml are produced from a single BC. Although contaminating leukocytes (about $3 \times 10^6$) are still present, the number of leukocytes in PC from BC is remarkably lower when compared to concentrates prepared via the PRP method ($20-200 \times 10^6$). Compared to the PRP method, platelets are less activated and the platelet recovery is higher [32].

For transfusion, usually five single PCs are pooled into a volume of about 300 ml. For the pooled BC method, 4-6 BCs and a unit of plasma or synthetic medium are pooled and centrifuged (soft spin). The resulting platelet-rich supernatant is transferred into a storage bag with high gas-permeability capacity. Before transfer to the storage bag, PCs may be filtered to remove residual leukocytes.
Pooled and single PCs are stored on a horizontal flatbed shaker at 22°C for a maximum of 5 days. BCs can also be pooled before preparation of PC. After adding plasma or platelet-additive solution, this pool is centrifuged at soft spin and the platelet-rich supernatant is transferred in special PC storage bags. When the pooled-BC method is used, the leukocyte concentration is lower and the platelet recovery higher compared to the single-BC method. PCs prepared via the pooled-BC method have to be stored on a horizontal flatbed shaker for a maximum of 5 days in special storage bags with an increased surface for gas exchange.

The main differences between PC prepared by the BC method and by the PRP method are listed in table 1.1.
**General Introduction**

<table>
<thead>
<tr>
<th></th>
<th>PC via PRP method</th>
<th>PC via pooled-BC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte number [33]</td>
<td>Approx. 5% of initial donor unit</td>
<td>Approx. 0.5% of initial donor unit</td>
</tr>
<tr>
<td>Platelet activation</td>
<td>More activation during first two days of storage and</td>
<td>Less platelet activation during storage</td>
</tr>
<tr>
<td></td>
<td>prolonged storage</td>
<td></td>
</tr>
<tr>
<td>Platelet yield [34]</td>
<td>60-75%</td>
<td>60-75%</td>
</tr>
<tr>
<td>Plasma yield [34]</td>
<td></td>
<td>75 ml more plasma yield than PRP method</td>
</tr>
</tbody>
</table>

**Table 1.1: Differences between BC en PRP-prepared PC**

1.6 **Risks of platelet transfusion**

Transfusion of platelets carries a risk of viral, bacterial, and protozoal transmission and may cause transfusion reactions due to immunomodulation, alloimmunisation, cytokines and/or platelet refractoriness.

1.6.1 **Viruses**

Viruses, such as cytomegalovirus (CMV), Human T-cell Lymphotropic Virus type I (HTLV I) and Epstein-Barr Virus (EBV), Hepatitis A, B and C (HAV, HBV, HCV), human immunodeficiency virus (HIV), can be transmitted via various blood products including platelet concentrates. Leukocyte filtration markedly decreases transmission of some of these viruses and is generally accepted as an alternative for CMV screening [35-37].

1.6.2 **Bacteria**

Due to storage of PC for a maximum of 5 days at 22°C and pooling, there is an increased risk of transfusion-associated infections caused by bacterial contamination. The following micro-organisms have been implicated: *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Bacillus cereus, Enterobacter cloacae, Streptococcus mitis, and Staphylococcus epidermidis* [38]. Bacteria can multiply during storage to high concentrations that can cause serious complications and even death in recipients. The reported incidence of bacterial contamination in PC varies from 0.1 to 2.5% depending on culture method used, type of PC (single or pooled units derived from whole blood or apheresis and shelf life at time of sampling) [39-41]. The incidence of transfusion-associated infections
proven to be caused by transfusion of bacterially contaminated PCs is much lower, i.e. in the range of 0.005% to 0.14% [42-44], again depending on type and age of PC used for transfusion. These data show that not all bacterially contaminated units lead to detectable transfusion reactions.

1.6.3 Infectious prion protein

Although it has never been proven, there is concern that the neurodegenerative Creutzfeld-Jakob disease (CJD) may be transmitted by blood or blood products. Besides CJD, there is a new variant (nvCJD) of this disease caused by the bovine spongiform encephalopathy (BSE) agent. This agent, called a prion protein (PrPSc, causing scrapie in sheep and goats), is transmitted by the consumption of meat of BSE-infected cows. Normal prion proteins (PrPC, conventional) are present at high levels in neurons and astrocytes within the central nervous system and at low levels in peripheral tissues, including reproductive tract, heart, lungs and lymphoid tissues, such as spleen, tonsils and appendix. Infection with PrPSc can turn normal PrP in PrPSc by conformational changes. Till now, transmission of PrPSc via blood or blood products has never been proven. In mice, differentiated B cells are crucial for neuro-invasion by scrapie, regardless of the specificity of their receptors. It is thought that these cells, which transport prions to the nervous system, do not need to express PrPC for uptake and transportation of prion protein [45;46]. In countries, such as the United Kingdom and Portugal, these findings have led to the introduction of routine leukocyte filtration of all blood components. In recent studies [47;48] however, no infectivity was detected in lymphocytes from peripheral blood. PrPC was expressed at low levels in lymphocytes and at higher levels in follicular dendritic cells, which do not reside in the peripheral blood. It was speculated that there is a possible role of the lymphoreticular system in processing PrPSc before the prions can spread to the central nervous system.

1.6.4 Protozoal transmission

Malaria and Chagas' disease are of greatest concern when protozoal transmission via blood transfusion is studied. In particular geographical areas or circumstances, babesia, leishmaniasis and toxoplasmosis may also offer risk. For more details of the latter three diseases, one is referred to Dodd [49].

Chagas' disease (Trypanosomaniasis) is caused by Trypanosoma cruzi, mainly found in South America and rarely in North America and Europe, and can be transmitted by blood products. Filtration can remove the protozoa to a great extent [50].

In addition, malaria can be transmitted by blood; therefore, infected individuals who have travelled in endemic areas will be a risk factor for blood donation in non-endemic areas. A one-year deferral for those who have travelled to malarious areas and a longer deferral (up to three years) for those who report a
General Introduction

history of malaria or those who have been resident in a malaria-endemic country can prevent possible transmission.

1.6.5 Cytokines

During PC storage the concentration of cytokines produced by white cells increases, notably in non-filtered PCs. Therefore, the incidence of febrile, non-haemolytic reactions is higher among recipients of non-leukocyte-reduced PCs stored for 4-5 days compared to those stored for 1-2 days. Muylle et al. [51] found high cytokine concentrations (IL-6 and TNF-alpha) in stored PCs, which, after transfusion, caused febrile reactions. These investigators also measured the effect of prestorage removal of leukocytes by filtration and BC removal in PC on the cytokine levels during storage [52] and concluded that this might prevent febrile transfusion reactions. Cytokines do not accumulate during storage of PC collected by apheresis, due to a low initial leukocyte concentration. Heddle et al. [53] found that cytokine concentrations (IL-1β and IL-6) increased during storage, which did not occur when PC were filtered. With respect to PC preparation, BC-PC is preferable over PRP-PC because of its low leukocyte concentrations and thus lower concentration of cytokines after storage [52].

1.7 Platelet refractoriness

Although there is no universally accepted definition of "platelet refractoriness", most authors use this term to indicate inadequate platelet increment upon two consecutive platelet transfusions in the absence of clinical factors known to affect the platelet response [54-57]. Factors such as platelet quality, non-alloimmune conditions and alloimmune factors influence platelet refractoriness. The PC quality depends on the number of platelets transfused, leukocyte contamination, storage duration, type of storage bags and temperature of storage. Non-alloimmune factors include platelet quality, fever, infection, disseminated intravascular coagulation, circulating immune complexes, bone marrow transplantation, splenomegaly, auto-antibodies and drug-related antibodies. HLA antibodies, platelet-specific antibodies and AB0 antibodies may evoke strong transfusion reactions with destruction of donor cells and febrile reactions of the patient. (For overview see Novotny [54]). Leukocyte filtration greatly reduces HLA allo-immunisation through transfusion of PC, although it cannot completely prevent platelet refractoriness [54-55]. The method for PC preparation also influences the incidence of non-haemolytic febrile transfusion reactions. Transfusion of PC prepared by the BC method causes significantly less non-haemolytic transfusion reactions compared to PRP-prepared PC [58]. When less than 5 x 10^6 leukocytes are present in PC, the formation of HLA antibodies drops to less than 5% of transfused patients [59;60]. However, for prevention of secondary HLA allo-immunisation, filtration of PC resulting in less than 5 x 10^6 leukocytes per PC is not effective [61].
Chapter 1

1.8 Filtration of platelet concentrates

Depending on the preparation method, PCs are contaminated with variable numbers of leukocytes, which can cause adverse reactions in patients. These adverse reactions can partially be prevented by leukocyte filtration. In some blood banks, this is a routine procedure for all PC, while in other blood banks PCs are only filtered when indicated. After April 1, 2000, all PCs prepared in The Netherlands will be filtered. Besides a higher cost aspect, a disadvantage of filtration is a loss of platelets due to the filtration step.

1.8.1 Removal of leukocytes

Leukocyte filtration of PCs reduces the formation of allo-antibody-mediated refractoriness to platelets given during intensive chemotherapy [54;55]. As mentioned above, antibodies against allo-antigens present on donor platelets and leukocytes are major causes of refractoriness to platelet transfusions in patients with thrombocytopenia. These antibodies usually arise in response to HLA class-I antigens on leukocytes and platelets, and less frequently from maternal-foetal incompatibility or repeated blood transfusions [54].

In The Netherlands, in single PCs of 40-70 ml, not a maximum of 50 x 10^6 leukocytes are allowed in at least 95% of all PCs. In pooled concentrates from 4-6 units, a maximum of 300 x 10^6 leukocytes is allowed. In filtered units (single as well as pooled PC) these concentrations should be less than 5 x 10^6 and preferably less than 1 x 10^6 in at least 90% of the PCs.

1.8.2 Loss of platelets due to filtration

During the filtration process, some platelets stick to the filter material or stay in the “dead volume” of the filter. These trapped platelets are not recovered in the filtrate and are lost for transfusion purposes [62-65]. The total loss, which usually is about 15%, depends on the number of filtered platelets, because after saturation of the filter, no more platelets adhere to the filter [66]. To minimise the interaction of the filter material and platelets, materials are modified to induce high platelet recovery.

1.8.3 Platelet compatibility of filter material

Platelets easily become activated by foreign surfaces [12]). By increasing the hydrophilicity or hydrophobicity or both, protein adsorption towards these surfaces can be minimised, leading to reduced adsorption of adhesive proteins and resulting in less platelet interaction with the filter surface [67]. Hydrophilic surfaces also induce less conformational changes of proteins that in turn give less platelet interaction. Despite low protein adsorption on hydrophobic surfaces, these surfaces are not suitable for filtration due to low wettability capacity. Another advantage of
hydrophilic surfaces is the pronounce adhesion of leukocytes [68-70]. Coating of non-woven poly(ethylene terephthalate) [NW-PET] surfaces with certain polymers prevents protein interaction and creates platelet-compatible surfaces under static conditions [67;71]. Under flow conditions, platelet compatibility is different, due to conformational changes of adhesive proteins as mentioned before (1;2). Under static conditions, for example Amiji used triblock copolymers consisting of poly(ethyleneoxide) [PEO] and poly(propyleneoxide) [PPO] chains in the form of PEO-PPO-PEO. The hydrophobic PPO middle part of the triblock copolymer adsors to the surface (glass) while the PEO parts, through a mechanism of steric repulsion, repels fibrinogen and platelets. A minimal length of the PPO part seemed to be essential for platelet compatibility. For more detailed information, see the introduction of chapter 5 of this thesis.

1.8.4 Possible hypotensive reactions after transfusion of filtered PC

In rare cases, patients develop hypotensive reactions to platelet suspensions filtered with a negatively charged white cell reduction filter. These reactions are mostly observed by using bedside filtration and in patients taking angiotensin-converting enzyme (ACE) inhibitor drugs [72-74]. ACE inhibitor inactivates enzymes that catabolise vasoactive substances such as bradykinin, which plays an important role in inflammatory reactions. It is a strong vasodilator, increases capillary permeability and stimulates nerve endings, thus producing pain [75]. Bradykinin is catabolised by ACE and has a plasma half-life time of only a few seconds. Filters containing electro-negatively charged fibre material may induce bradykinin production in plasma-containing platelet and red cell concentrates, by triggering kallikrein formation after the activation of prekallikrein activator (PKA = FXIIa) [76]. Due to the rapid destruction of bradykinin and the neutralisation of PKA by Cl-esterase inhibitor, prestorage filtration of PC can prevent possible hypotensive reactions caused by bradykinin. This is one of the reasons to prefer prestorage filtration to bedside filtration as well as to prefer artificial crystalloid storage media to plasma. [77;78].

1.9 Quality control of stored platelet concentrates

During storage, the quality of PC and the composition of the storage medium undergo changes that limit the usage of PC for transfusion. Furthermore, the survival of platelets in vivo is short [79;80]. These are two of the main reasons why platelets are stored for a maximum of 5 days.

The quality of PCs can be tested in various ways. First, the degree of platelet activation can be measured, and depends on factors such as storage medium, storage duration, storage bag, storage temperature and leukocyte contamination. During the platelet activation process, concentrations of intra- and extracellular metabolites change and granular contents are gradually released. Furthermore,
expression of membrane antigens and concentrations of blood gasses (O_2 and CO_2) in the storage medium change from day to day. The increase in CO_2 induces a drop in pH. It is not known which of these in vitro parameters correlates best with in vivo survival of stored platelets. A combination of activation parameters will be the best choice [81]. A literature study from the BEST Task Force revealed the hypotonic shock response, morphology score according to Kunicki, extent of shape change and swirling ("shimmering") have stood the test of time, and many laboratories have used them successfully. Measurements for quality control which are widely used include: platelet concentrate volume, platelet count, pH at completion of storage, determination of swirling and leukocyte count when the aim is to prepare a leukocyte-depleted product [82]. Some parameters, such as pH and swirling, can easily be measured before transfusion of PC. Moreover, a rapid drop in the pH may indicate the growth of bacteria.

1.10 Purpose of the studies described in this thesis

The goal of the study described in this thesis was to optimise the quality and safety of PC for transfusion by improving methods for the preparation, leukocyte filtration and storage of PC and by developing sensitive methods for monitoring the quality of PC.

For various reasons, the presence of leukocytes has deleterious effects on the quality of PC and the clinical effectiveness of PC transfusions. Although the number of leukocytes in some of the currently used PC (BC-PC) is low, the degree of contamination is still too high to guarantee optimal quality.

In this thesis, a preparation method is described to achieve lower leukocyte counts in PC without using filtration. A combination of optimal centrifugation, buffy-coat size and composition and shape of the BC bag has been tested.

To achieve optimal leukocyte depletion of PC, NW-PET filter material with good leukocyte-depletion properties was modified. Two different techniques were tested, a dry-chemical method (radio frequent glow discharge process (RFGD) [70]) and a wet-chemical process (coating with different polymers). These modified materials were tested in a mini filter that allowed the measurement of leukocyte and platelets recoveries.

Good gas exchange of the PC storage bags, which is essential for platelet survival, is one of the most important factors for platelet storage. We compared the effects of storage conditions on PC in special PVC bags with long chain plasticizer and in polyolefin plastic bags. This was done by measuring platelet metabolism and platelet activation, as well as the influence of increased oxygen and carbon dioxide exchange properties of this new storage container.

Sensitive platelet activation assays, such as the platelet morphology score and β-thromboglobulin (β-TG) content in the supernatant of the PC, which are currently used to monitor the quality of PC, have certain disadvantages. In this thesis, two ELISA assays for activation markers (P-selectin and glyocalcin) in the
supernatant of PC are described that overcome some of the limitations of the current assays. The sensitivity of these ELISA assays has been compared to the existing platelet activation assays.