From the Division of Clinical Immunology and Transfusion Medicine at the Department of Laboratory Medicine
Karolinska Institutet, Stockholm, Sweden

STORAGE AND TRANSFUSION OF PLATELETS: IN VITRO AND IN VIVO STUDIES IN HEALTHY VOLUNTEERS AND IN ALLOGENEIC HEMATOPOETIC PROGENITOR CELL TRANSPLANT RECIPIENTS

Beatrice Diedrich

Karolinska Institutet

Stockholm 2009

Published by Karolinska Institutet. Printed by Alfa Print AB, Box 6003, 171 06 Solna

ISBN 978-91-7409-280-6

To my family

ABSTRACT

Early studies in the 1960´s showed that platelet (PLT) transfusions significantly reduced the incidence of fatal hemorrhages. Together with the advances in the hematology and transplantation field, easily accessible PLTs for transfusion use have resulted in a rapid increase of PLT utilization and a change from therapeutic -i.e., treatment of clinical bleeding- to prophylactic PLT transfusions, despite limited clinical evidence regarding whether a prophylactic PLT transfusion strategy results in a lower morbidity or mortality compared with transfusing patients with early signs of bleeding. About 2 million PLTs are transfused in the United States, 2.9 million in Europe and 40 000 in Sweden with a roughly ten-fold increase since 1984. In a 1991 survey in the United States, more than 70% of hospitals reported transfusing PLTs primarily for prophylaxis. Continued increase in demand may result in problems with shortages and difficulties to comply with orders.

Several in vitro studies have shown satisfactory quality results for platelets stored for 7 days and even longer. Storage has been limited to 5 days because of the risk of bacterial contamination, with subsequent bacterial increase during storage at room temperature. Provided that contaminating bacteria can be effectively detected or put out of action in platelets, prolonged storage represents one possibility to obtain improved availability, logistical management and decreased outdating.

The overall aim of this thesis was to assess the quality of platelets after prolonged storage up to 7 days and to evaluate the consequences of lowering the platelet transfusion trigger for prophylactic transfusion, representing two possible approaches suggested to have impact on the platelet accessibility.

In paper I, we showed that a prophylactic platelet transfusion trigger level of 10 instead of 30 $x10^9$ platelets/L for allogeneic HPCT recipients considerably reduced the number of PLT transfusions without increasing the incidence of hemorrhagic events. In paper II, recovery and survival in healthy volunteers of PLTs stored for 7 days decreased, but met the suggested criteria. Analyzed in vitro parameters showed acceptable results. Addition of potassium and magnesium to the storage medium indicated higher quality in vitro but this could not be verified by in vivo recovery and survival (paper III). In paper IV, when comparing transfusion in allogeneic HPCT recipients of PLTs stored for 6-7 days vs 1-5 days, a significant lower platelet increment (CCI) and shorter interval between transfusions was noticed with prolonged storage.

To have sufficient PLTs of excellent quality in stock at any given time to provide to patients in great need represents a difficult challenge to transfusion services. If prolonged storage results in decreased time between the transfusion and more PLTs are transfused in general –the result might not be what was aimed at. In that case it might be wiser to question the need of prophylactic transfusion.

Lately it has been proposed that patient selection may be the key to the safety of therapeutic only based platelet transfusion strategy.

To conclude, there is a growing need of further randomized, controlled clinical trials to establish the therapeutic approaches that maximizes the quality of patient care in different patient categories while minimizing expenses to transfusion services and the health care system to be able to ensure an adequate PLT supply when really needed.

LIST OF PUBLICATIONS

I. **Diedrich B**, Remberger M, Shanwell A, Svahn B-M, Ringdén O. A prospective randomized trial of a prophylactic platelet transfusion trigger of 10 vs 30 x109/L in allogeneic hematopoietic stem cell transplant recipients. Transfusion 2005;45:1064-1072.

II. Shanwell A, **Diedrich B**, Falker C, Jansson B, Sandgren P, Sundkvist L, Svensson L, Vesterinen M, Gulliksson H. Paired in vitro and in vivo comparison of apheresis platelet concentrates stored in platelet additive solution for 1 versus 7 days. Transfusion. 2006;46:973-979.

III. **Diedrich B**, Sandgren P, Jansson B, Gulliksson H, Svensson L, Shanwell A. In vitro and in vivo effects of potassium and magnesium on storage up to 7 days of apheresis platelet concentrates in platelet additive solution. Vox Sang. 2008;94:96-102.

IV. **Diedrich B**, Ringdén O, Watz E, Shanwell A. A randomized study in allogeneic hematopoetic progenitor cell transplant recipients of prophylactic transfusion of buffy coat platelets stored for 1-5 versus 6-7 days in platelet additive solution. Submitted (Vox Sang).

CONTENTS

LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BC	Buffy coat
CCI	Corrected count increment
CFU	Colony forming unit
CNS	Central nervous system
ESC	Extent of shape change
GP	Glycoprotein
GVHD	Graft versus host disease
HLA	Human leukocyte antigen
HPA	Human platelet antigen
HPC	Hematopoetic progenitor cell
HPCT	Hematopoetic progenitor cell transplant
HSR	Hypotonic shock response
LDH	Lactic dehydrogenase
MoAb	Monoclonal antibody
MPV	Mean platelet volume
PAS	Platelet additive solution
PF4	Platelet factor 4
PLT	Platelet
PRP	Platelet rich plasma
RANTES	**R**egulated on **A**ctivation, **N**ormal **T** cell-**E**xpressed, and **S**ecreted
RBC	Red blood cell
VOD	Veno occlusive disease
WB	Whole blood
WBD	Whole blood derived
WHO	World Health Organization

1 INTRODUCTION

1.1 PREPARATION AND STORAGE OF PLATELETS

1.1.1 Brief historical background

The value of transfusing platelets (PLTs) in thrombocytopenic patients with bleeding disorders was already reported in 1910 when Duke described three patients with hemorrhages due to thrombocytopenia, each of whom showed improvement after transfusion of fresh whole blood (1). At that time PLTs were administered by direct transfusion of whole blood (WB) without an anticoagulant, later citrated fresh whole blood collected in glass bottles was used.

In the 1960´s, plastic containers replaced the breakable glass bottles. This development made it possible to prepare platelet rich plasma (PRP). Four to six fresh PRP units were considered as a suitable transfusion dose and the benefit of PLT transfusions in patients treated with chemotherapy for leukemia was shown in several studies by the reduced incidence of fatal hemorrhages (2, 3).

During the 1970´s and 1980´s advances regarding the storage condition for PLTs were made as Scott Murphy and coworkers demonstrated the possibility to store PLTs at room temperature for several days (4-6). Further improvements during the last decades including the introduction of platelet additive solutions (PAS) have made it possible to store PLTs >5 days (7). At the present time, the possibility of 10- and even 12-day storage may be approaching (8).

1.1.2 Bacterial contamination

The benefit of storing PLTs at room temperature has however the disadvantage of facilitating bacterial overgrowth with associated risk of clinical septicemia, one of the main risks of transfusion transmitted diseases (9). Therefore storage of PLTs is limited to 5 days but may be prolonged to 7 days if detection or reduction of bacterial contamination is assured (10). Bacterial contamination of blood components derives from the introduction of low concentrations of skin bacteria at the time of phlebotomy due to ineffective disinfection, or during the preparation of blood components if the sterility chain fails or but less commonly, from asymptomatic donor bacteremia.

Although bacterial contamination affects a “small” proportion of PLTs (about 1 in 1,000 to 2,000 PLTs (11)) it may be fatal, particularly in immuno-compromised patients with cancer or blood disorders. At present, two strategies are applied to limit the risk of transfusion-associated sepsis involved with prolonged storage of PLTs, bacterial detecting tests and pathogen inactivation.

Several methods have been developed to screen for contaminated PLTs and some judged not enough sensitive have been abandoned. Automated liquid media culture capable of detecting low levels of bacteria (≥10 CFU per mL) and a system that monitors the fall in oxygen tension as an indication of bacterial growth (with a threshold for detection of $\geq 10^{2}$–10^{3} CFU per mL), have reduced but not eliminated the risk of bacterial contamination (12).

The other strategy, pathogen inactivation, uses nucleic-acid intercalating agents such as psoralens or riboflavin which, in the presence of light, bind to nucleic acids of pathogens and inactivate them by inhibiting replication, while permitting the nucleic-acid-free constituents of donor blood (plasma, platelets and RBCs) to continue to function (13). These technologies can eliminate most of the risk of bacteria. However regarding amotosalen treated platelets, some concerns exists about reduced in vivo recovery and survival, reduced corrected count increment (CCI) and shorter inter-transfusion interval although adequate hemostasis seem to be provided (14-19).

1.1.3 Techniques for platelet preparation

The PLTs can be prepared from whole blood using either the platelet rich plasma (PRP) or the buffy coat (BC) method. Another technique in use is apheresis. In the United States, PLTs derived from whole blood are produced by the PRP method whereas the BC method is used in Europe. The use of apheresis derived PLTs has increased to approximately 75-80% in the USA and 50% in Europe (20). In Sweden around 35-40% of PLTs are apheresis derived (Transfusion services in Sweden 2007: extent, quality and safety. Swedish Society for Transfusion Medicine).

For the preparation of PRP, collected whole blood is centrifuged at a slow speed (soft spin, approx 500 *g*) that sediments the red and white cells and concentrates most platelets in the supernatant plasma. A second higher speed (hard spin, approx 3000 *g*) is then applied to the platelet rich plasma which sediments the platelets. The supernatant plasma (platelet free) is removed and sedimented platelets are resuspended in a small portion of plasma. Four to six concentrates, each containing on average 70×10^{9} platelets and $< 0.2 \times 10^{6}$ leukocytes in 90% of units (leukoreduced product), are necessary to obtain what is generally considered as an adult “standard dose” of recovered platelets (10).

Contrary to the PRP method the first centrifugation step in the BC method is a high speed of the collected whole blood resulting in a platelet rich buffy coat. Four to six BCs are pooled in a pooling container (often with a storage medium) and centrifuged by a slow speed to a platelet rich supernatant that is transferred to the storage container and with platelet content as above (7, 21).

The apheresis method involves only one single donor, which is an advantage compare to the techniques above where several donors are needed, if platelets lacking a certain antigen property are needed for an immunized patient. Another

advantage is the reduction of numbers of donors to whom a patient is exposed to because it reduce the risk of transmission of any transfusion-transmitted agent known today as well as others that could emerge in the future. In this procedure, whole blood from a donor is processed with a cell separator with an inline centrifuge for platelet collection. The platelets are transferred to a collection bag whereas the red blood cells (RBCs) and most of the plasma are returned to the donor. On the market there is a variety of cell separators with different collection principles (22). Standard total platelet count required per unit is > 200x 10^9 and leukocyte count < 1 x 10^6 (leukoreduced product) in ≥ 90% of units (10).

1.1.4 Storage of platelets.

For several reasons, storing PLTs in synthetic media is becoming a more popular practice and is currently a standard in several European countries. The possibility to increase the availability of plasma for other important causes and to diminish the risk of transfusion reactions is some arguments among others (23). For the long-term storage of PLTs, synthetic media also offers the possibility to add key components in the storage medium such as glucose, acetate, citrate, phosphate, potassium and magnesium in order to optimize the storage conditions as regards the platelet metabolism and possibly also for the reduction of platelet activation (7, 24).

An important aim in transfusion medicine is to ensure an adequate supply of PLTs of optimal quality for transfusion. Due to the uneven demand and the short storage period of PLTs, there are sometimes PLTs shortages, especially during extended holiday periods. Provided that contaminating bacteria, as discussed above, can be effectively detected or put out of action in PLTs, prolonged storage offers the advantage of improved availability, logistical management and decreased outdating (25).

The effect of extending storage > 5 days has shown promising results on in vitro parameters (7, 8, 26-28) and on in vivo post transfusion recovery and survival of autologous platelets in healthy volunteers (29, 30). Only limited data are available on the effects of prolonged storage time on posttransfusion recovery in patients and are difficult to compare since both the selected patient groups and the methods used to prepare the PLT products varies substantially (31, 32). To provide additional verification of the suitability of PLTs stored > 5 days, further randomized patient transfusion studies are needed.

In order to maintain viability platelets must continuously generate ATP to meet their energy needs. Data suggests that the major part (85%) of ATP production in PLTs during storage is derived from oxidative metabolism via the tricarboxylic acid cycle with fatty acids as substrate and carbon dioxide as end product. The other 15 % is generated via anaerobic glycolysis generating lactate and hydrogen ion which is buffered by bicarbonate in the plasma and converted to water and carbon dioxide (28, 33). In 1990 acetate was proposed as a substrate in the oxidative metabolism (34). Acetat was shown to reduce lactate generation and

an additive solution was designed (35). For the long-term storage in PAS, besides the importance of reducing the metabolic rate and the platelet activation during collection, preparation and storage, glucose also needs to be available in the storage medium during the entire storage period (7).

Several factors besides the platelet additive solution influence the storage of PLTs i.e. the preparation method, the plastic composition of the storage bag, the agitation of PLTs during storage and the temperature to mention some.

Lack of oxygen is detrimental to platelet metabolism so the ability of the storage bag to permit gas exchange (oxygen and carbon dioxide) is crucial. The gentle agitation to prevent the platelet sedimentation and enhance the transport of oxygen and carbon dioxide is also of importance (36). Early storage bags had poor oxygen permeability and storage was only allowed for up to 3 days. The aerobic metabolism could not be maintained which resulted in lactic acid production and a fall in pH (5). Newer plastics have shown good gaseous permeability and results in good in vitro and in vivo viability for 7 days (29).

The advantage of lowering the temperature from physiological 37°C to 22°C is to obtain a much lower energy metabolism and a temperature around 20-24°C has been found to be optimal for the storage of PLTs (37).

Albeit the currently accepted optimal status for platelet storage is used, platelet viability and function declines over time, a phenomenon known as the “platelet storage lesion”(38). Numerous morphologic, biochemical and functional derangements occur during collection, processing and storage and there is considerable interest in finding a method for its prevention.

1.2 QUALITY TESTING OF PLATELETS

Extending the storage period of PLTs will probably have some effect on platelet quality and there is considerable interest in studying the quality of PLTs stored for more than 5 days, but presently, there is no general agreement on the type of criteria that should be applied to stored PLTs (31).

Essentially, there are three different ways to study the quality of PLTs namely, testing platelet function and metabolism in vitro, in vivo recovery and survival of labeled autologous platelets in healthy volunteers, and platelet transfusions in thrombocytopenic patients.

While a variety of methods have been applied to evaluate platelet quality during storage (39), there appears to be no in vitro test that clearly predicts the in vivo recovery, survival and function of transfused platelets. However, a battery of in vitro tests reflecting the different aspects of platelet function is widely used in an attempt to identify the platelet storage lesion by detecting changes that occur

during collection, processing and storage of PLTs, and usually precedes or comes together with in vivo tests when assessing platelet quality.

This difficulty of predicting the outcome of platelet transfusion in vivo from the results of laboratory testing, in particular using single in vitro parameters, is well known (39-44). Some in vitro criteria are although crucial. Swirling, a phenomenon obtained by the reflection of light when discoid platelets are exposed to a light source, should be present, because its absence is highly predictive of poor posttransfusion platelet count increments; pH should be in the range of 6.4–7.4, and glucose should be present in the PLTs throughout storage as energy fuel (7, 10, 41, 45, 46). PLT ATP levels should be >4.0 μmol/10^{11} /PLTs since lower concentrations may be associated with loss of in vivo viability (47).

Usually a panel of assays able to measure platelet activation and function is also applied in an attempt to evaluate the platelet storage lesions of which someones will briefly be mentioned here (48). Hypotonic shock response (HSR) has been shown to be a suitable in vitro test to predict posttransfusion efficacy (49, 50). An in vivo recovery of < 50% appears to correspond to an HSR of < 70-75% of fresh platelets (49). RANTES, stored in platelet α-granule, is released into the PLTs when platelets are activated (41). An increase during storage has been noted (31) but the clinical significance is unclear and the levels at day 7 are lower than the concentration reported to be associated with allergic reactions (51). P-selectin (CD62p), a membrane protein released from α granule onto the cell surface of the platelet upon activation, is frequently used as a marker of platelet activation, yet like RANTES, its clinical relevance is uncertain. It has been suggested that increased rate of clearance of platelets from the circulation is correlated to increased expression of P-selectin but that loss of in vivo viability first occurs with relatively high levels of platelets positive for P-selectin (above 50-60%) (41). Michelson et al also 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 (52). Test of platelet function can also include methods that measures clot formation and retraction but that is beyond the scope of this thesis.

In vivo studies with radionuclide labelled autologous platelets in healthy subjects are considered to be the best assessment of platelet quality (39). They are informative but expensive, complex to perform and imply hard work for the donor (time-consuming, sampling, ethical aspect with radionuclide etc). An aliquot of platelets is labelled and reinfused to the corresponding donor and samples are taken at different point of time. The radioactivity is measured and survival and recovery can be calculated and estimated. Because of the great variation in results among normal donors as regards recovery and survival it is advised to perform paired platelet studies where each individual represents its own control. Each donor thereby tends to give a high or low value in both study arms (53). A standard has been proposed which states that the mean recovery should be >66%of fresh platelets and survival> 50% of fresh platelets (54).

Finally, most relevant data comes from clinical studies in thrombocytopenic patients but the endpoint is most frequently the post transfusion increment, which does not provide much information on platelet function, although to be able to function the platelets must circulate. There are several methods of determining the increment: count increment ((CI) postransfusion minus pretransfusion platelet count), corrected count increment (CCI) and percent platelet recovery (PPR). The latter two require knowledge of the number of platelet transfused and an estimate of patient's body surface area or blood volume respectively.
However, most studies are performed where PLTs are ordered for prophylaxis, which is different to their use for the treatment of acute hemorrhages. In the case of hemorrhages, the primary role of platelets is to adhere to the damaged endothelium and form a haemostatic plug. Consequently it is supposed that many functional platelets are not present in the circulation for sampling under these circumstances and survival is of less importance. This explains some of the difficulties associated with assessment of platelet quality and why the results in stable nonbleeding patients cannot be extrapolated to bleeding patients.
To assess hemostatic function of platelets, bleeding as an outcome measure is nowadays often used and the World Health Organization (WHO) Grade 1-4 bleeding scale is applied (55). WHO Grade 0 is no evidence of bleeding; WHO Grade 1 is the mildest form of bleeding characterized by petechia, ecchymosis and mucosal bleeding; WHO grade 2 is gross bleeding including melena, hematemesis, hematuria or hemoptysis; WHO Grade 3 includes any bleeding that requires RBC transfusions; and WHO Grade 4 includes retinal bleed with visual impairment, cerebral bleeds associated with morbidity and fatal bleeds. Most clinicians consider platelet transfusions necessary for patients who develop WHO bleeding grades ≥ 2 (56).
But there are many difficulties to deal with (57) including grading and no general agreement over which grades are the most relevant endpoints. For severe bleedings that occurs rarely, a large number of patients is often needed.

1.3 CLINICAL USE OF PLATELETS

1.3.1 Platelet structure and life cycle

Platelets are small and discoid in shape, averaging only 2.0 to 5.0 μm in diameter and 0.5 μm in thickness. Platelets are anucleated, derived from bone marrow megakaryocytes and contain three major types of secretory organelles namely; α-granules (containing glycoprotein receptors and matrix adhesive proteins), dense bodies (containing ADP, Ca^{2+} and nucleotides) and lysosomes. Platelets have an open canalicular system of internal membranes formed into a network of tubules that permeates the platelet. When platelets become activated the canalicular system serves as a channel into which the platelet granules fuse and release their contents. Platelets express HLA-A and HLA-B, human platelet (HPA) and ABO blood group antigens and antibodies against these may influence the results of PLT transfusions (56).

Platelets circulate in a concentration of 150-400 x 10^9/L with a normal lifespan around 7-10 days. The clearance of old platelets occurs primarily in the spleen and liver by macrophages which accounts for the major loss in healthy individuals together with the fixed number, approximately 7100 platelets/μL per day, required for maintenance of the vessel integrity (58).
Another loss of platelets occurs following damage to the vessel wall when subendothelial collagen, von Willebrand factor or fibronectin activates them. The platelets then adhere to the damaged site, undergo shape change, secretion and aggregate to form a platelet plug. The platelet plug is thereafter stabilised by fibrin formed in the coagulation cascade. If there is a defect in any of these platelet functions or and/or in the number of platelets, hemostasis might be impaired with an increased risk of bleeding.

1.3.2 Platelet transfusion therapy

Early studies in the 1960´s showed that platelet transfusions significantly reduced the incidence of fatal hemorrhages (2, 3) and today, it is uncommon for a patient undergoing intensive chemotherapy to die of hemorrhage. Together with the advances in the hematology and transplantation field, easily accessible platelets for transfusion use have resulted in a rapid increase of platelet utilization and a change from therapeutic -i.e., treatment of clinical bleeding- to prophylactic platelet transfusions. In a 1991 survey in the United States, more than 70% of hospitals reported transfusing platelets primarily for prophylaxis (59). About 2 million PLTs are transfused per year in the United States (60), 2.9 million in Europe (12) and 40 000 in Sweden with a roughly ten-fold increase since 1984 (Transfusion services in Sweden 2007: extent, quality and safety. Swedish Society for Transfusion Medicine). Continued increase in demand may result in problems with shortages and difficulties to comply with orders.

With the intention to prevent hemorrhages, frequent prophylactic platelet transfusions are given to keep the patient's peripheral blood platelet count above a predetermined threshold. The threshold is selected because of clinical concerns that the patient is at a higher risk of morbidity or mortality due to bleeding if their platelet count falls below a defined level (the trigger). However, the level of the threshold has been a matter of long-standing debate (61). Traditionally, the transfusion trigger has been set at 20 x10^9 platelets/L on the basis of a study done in the early 1960s, which showed that severe hemorrhages rarely occurred in patients above this limit, in spite of widespread aspirin use (62). In recent years, a lower "safe" threshold has been reported in patients with various conditions (63-71) and in 2004, the authors of a Cochrane systematic review concluded that there were no reasons to change the current practice of transfusion trigger 10x 10^9/L for prophylactic platelet transfusions (72). Lowering the threshold has been associated with a significant decrease in the use of platelet transfusions (63, 66-68, 70)

The threshold for prophylactic platelet transfusions is generally higher for surgery patients. Most consider a platelet count of > 50 x 10^9/L as adequate and in CNS procedures the recommended platelet count threshold is >100 x 10^9/L (73). A direct relationship has been shown between the platelet count and platelet survival in patients with platelet counts less than 100 x 10^9/L (58). When platelets are transfused for prophylaxis the platelets need to circulate and function for as long as possible. On the contrary when platelets are transfused because of ongoing hemorrhage an immediate hemostasis effect is needed and the survival time might be less important (39).

While no one would argue about the need of therapeutic transfusions for the treatment of bleeding complications in patients with hypoproliferative thrombocytopenia due to cytotoxic therapy or malignancies of the bone marrow, there are ongoing discussions about whether prophylactic transfusions are effective in reducing the risk of bleeding in clinically stable patients (74-76).

In thrombocytopenic patients there is not a clear association between the occurrence of a major bleeding episode and the platelet count. In a retrospective review there were no relationship between first morning platelet count or lowest platelet count of the day and the risk of hemorrhage (77). Patient factors associated with a greater risk for severe bleeding in this study were; a history of recent bleeding (previous 5 days), uremia, a recent bone marrow transplant and hypoalbuminemia (77). Such findings have led to the questioning of prophylactic transfusions in stable selected patients as opposed to the use of only therapeutic transfusions when clinical bleeding is present (75). In another review of case reports with intracranial hemorrhage in prophylactic transfusion studies no clear association with platelet count prior to the hemorrhage could be observed (76).

A recent study in autologous HSCT patients suggests that therapeutic based platelet transfusion strategy is safe (78). Preliminary results from a randomized trial in the same patient category in which treatment based versus prophylactic platelet transfusion are compared, therapeutic transfusions strategy seems safe and reduces platelet use (Wandt H. A Therapeutic Platelet Transfusion Strategy without Routine Prophylactic Transfusion Is Feasible and Safe and Reduces Platelet Transfusion Numbers Significantly: Preliminary Analysis of a Randomized Study in Patients after High Dose Chemotherapy and Autologous Peripheral Blood Stem Cell Transplantation**.** Blood (ASH Annual Meeting Abstracts), Nov 2008; 112**:** 286.

Lately it has been proposed that patient selection may be the key to the safety of therapeutic only based platelet transfusion strategy (74).

Another important question is the optimum dose of platelets, which has not yet been defined. Most centres use a standard dose of 300-600 x 10^9 platelets, which is about 5-10 x 10^9 platelets per kg of recipient bodyweight (73). However, in one recently completed study of prophylactic platelet transfusion with trigger 10x 10^9/L conducted by Sherill Slichter, (the PLADO trial), comparing standard dose (2.2 x10^{11} platelets/m^2) with lower dose (1.1 x 10^{11} platelets/m^2) as well as higher (4.4 x 10^{11} platelets/m^2), preliminary results indicates no difference in bleeding events or grades for the different regimes.(Effects of Prophylactic Platelet (Plt) Dose on Transfusion (Tx) Outcomes (PLADO Trial). Blood (ASH Annual Meeting Abstracts), Nov 2008; 112: 285.
These results suggest that smaller doses are as efficient as higher but since the intravascular lifespan is shortened at low platelet counts (58), higher doses could have advantages. In another study, comparisons high dose with standard dose, high dose resulted in greater post transfusion platelet count increment and increased intertransfusion interval without an increase in quantity of platelets transfused (79). Longer intertransfusion interval (fewer transfusion event) is more convenient for the recipient, especially for outpatients, and might cut the cost of administering the platelets. However it has been suggested that the period of thrombocytopenia may be shorter for patients who receive fewer platelets and/or who are maintained at a lower mean platelet count (80).

In a recently published paper comparing low-dose (150-<300 x 10^9 platelets) and standard-dose (300-600 x 10^9 platelets), a higher WHO grade 4 bleeding in low-dose group, 3/58 patients versus none/61 in standard-dose, resulted in this trial being stopped due to predetermined safety measures. Whether the difference was due to chance or not could not be determined. The proposed benefits of a low-dose strategy (fewer products being transfused and a shorter duration of thrombocytopenia) could not be verified either, however overlapping between the two groups as regards actual platelet dose was also present (81).

Finally to sum up, blood products including platelets may become an increasingly scarce resource in the future and there is a growing need of further randomized, controlled clinical trials to assess the optimal indications of platelet transfusions therapy in different patient categories.

2 AIMS

The overall aim of this thesis was to assess the quality of PLTs after prolonged storage up to seven days and to evaluate the consequences of lowering the platelet transfusion trigger for prophylactic transfusion, representing two possible approaches suggested to have impact on the platelet accessibility.
This aim was split up in the following papers to

Paper I
assess the implication of a lower platelet transfusion trigger in the prophylactic transfusion setting in allogeneic hematopoetic progenitor cell transplant recipients.

Paper II
evaluate the quality of PLTs after prolonged storage by in vitro and in vivo studies in healthy subjects

Paper III
investigate the significance of adding magnesium and potassium to the storage medium to optimize the quality of PLTs after prolonged storage by in vitro and in vivo studies in healthy volunteers.

Paper IV
determine the effect of prophylactic transfusion in allogeneic hematopoetic progenitor cell transplant recipients of PLTs after prolonged storage.

3 HEALTHY VOLUNTEERS/PATIENTS AND MATERIALS/METHODS

A summary of patients, healthy volunteers, materials and methods used is given below. For additional details, the reader is referred to the respective papers.

3.1 HEALTHY VOLUNTEERS (PAPER II AND III)

For the studies in healthy volunteers, autologous double apheresis PLTs from regular blood donors were collected using using the Trima blood cell separator (Gambro BCT, Lakewood, CO). The PLTs from each apheresis donor was divided equally and stored in two separate containers and used for a paired comparison.

In paper II, the quality of PLTs from a donor (n=10) stored for 7 days versus 1 day in T-Sol PAS (Baxter, La Châtre, France) was compared. T-Sol was added to the PLTs after the collection, (on day 0), with a plasma:T-Sol ratio of 40:60**.** Each half unit of PLT contained $312\pm88 \times 10^9$ platelets (mean ± SD). One unit was used on the day following the apheresis (= day 1), and the other, on day 7.

In paper III, the comparison was between the quality of PLTs from a donor (n=10) stored for 7 days in two different PAS. After collection, SSP+ (MacoPharma, containing potassium and magnesium) was added to one of the bags and Intersol (Baxter, without these additives) to the other. Platelet content approximately same as above.

3.1.1 In vitro studies

Metabolic and cellular parameters
Platelet counts and mean platelet volume (MPV) analysis were performed using a cell counter (Medonic, Cellguard CA 620, Boule Nordic AB Stockholm, Sweden). Enumeration of residual white blood cells (WBCs) in the PLT was performed with a Nageotte chamber (82).
Levels of pO_2 and pCO_2, and pH, bicarbonate, lactate, and glucose values were measured on a blood gas analyzer (ABL 705, Radiometer, Copenhagen, Denmark). Lactic dehydrogenase (LDH) was analyzed by spectrophotometry (DMS 100, Varian Analytical Instruments, Springvale, Australia). Concentration of total adenosine triphosphate (ATP) (µmol per 10^{11} platelets) was determined by luminescence technique (Orion Microplate Luminometer, Berthold Detection Systems GmbH, Pforzheim, Germany) (83).

Hypotonic shock response (HSR) and the extent of shape change (ESC) (paper III) were performed using the SPA2000 (Chronolog, Havertown, PA, USA), with the modifications of these test described by VandenBroeke et al. (84). Swirling was graded by inspection in accordance with Bertolini´s method (85).

Cytokine analysis
The plasma concentrations of RANTES and PF4 (paper II) were analyzed using commercial ELISA kits in accordance with the manufacturers' recommendations (Quantikine R & D Systems, Abingdon, UK).

Flow cytometry analysis (paper III)
A FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) was used for analysis of CD41, CD42b, CD61, CD62p and CD63 expression on PLTs as been described by Sandgren in earlier work (86), using MoAbs from Immunotech Beckman Coulter, Marseilles, France).

3.1.2 In vivo recovery and survival studies with radiolabelling

The wide variation between individual donor recoveries and survival times requires paired comparisons where each donor represents its own control (53, 87).

In paper II, PLTs from one of the two units were labeled with ^{111}Indium-oxine solution (DRN 4908 Indium oxinate; Mallinckrodt Diagnostica BV, Petten, The Netherlands) (approx half life 2,8 days) on day 1 and PLTs from the other unit were labeled with ^{111}Indium after 7 days storage. A dose of ≈ 2 MBq of ^{111}Indium-labeled platelets (approximately 10^{10} platelets) were infused through a peripheral vein of the corresponding donor. Samples from the donor were drawn 30, 60, and 150 minutes after injection, thereafter, once a day 7 days after the second injection. The ^{111}Indium activity in all samples and in the standard sample was counted in a gamma counter (Wallac 1282 Compugamma CS) (at 171 and 245 keV). All samples from the same donor was counted at the same time, with appropriate correction for the physical decay of ^{111}Indium during the counting sequence.

In paper III, donors randomized to even or uneven numbers. To be able to distinguish between the platelets stored in different PAS in the same individual, two different radionuclide were used, ^{111}Indium and ^{51}Cr ($Na_2{}^{51}CrO_4$; GE Healthcare Ltd., Little Chalfont, UK.) (approx half life 27,7 days). On day 7, platelets from donors with uneven number stored in Intersol were labeled with ^{111}In and the platelets stored in SSP+ were labeled with ^{51}Cr and vice versa for donors with even number. After labeling, a dose of ≈1 MBq ^{111}Indium -labeled platelets (around 10^{10} platelets) respectively ^{51}Cr -labeled platelets from the two units were injected through a peripheral vein of the corresponding donor. Blood samples from the donor were drawn 30, 60, and 120 minutes after injection (day 7). Thereafter, samples were drawn once a day for the following 8 days (day 8-16). The ^{111}Indium activity was counted as above and the ^{51}Cr activity was counted at 286-398 keV.

3.2 PATIENTS (PAPER I AND IV)

3.2.1 Paper I

Patients undergoing allogeneic HPCT at Huddinge University Hospital were randomized, after stratification for type of donor (related/unrelated), origin of stem cells (bone marrow/ peripheral blood stem cells (PBSC)) and age (children <18 years vs. adults), to receive prophylactic platelet transfusions when their morning platelet counts fell below $10x10^9$/L (group T10) or 30 $x10^9$/L (group T30). Patients with a known bleeding disorder or coagulopathy were excluded. The number of PLT units, RBC transfusions (units) and type of bleeding ≥ WHO grade 2 (-i.e., World Health Organization criteria: grade 0, none; grade 1, petechial; grade 2, mild blood loss; grade 3 to 4, gross or debilitating blood loss) (55), were recorded starting 7 days before and until 30 days after transplantation. PLT independence was defined as the first of seven days with platelet counts above the trigger level without transfusions.
Nurses from the ward, blinded to treatment arm, performed daily (inpatients) or twice a week (outpatients) the assessment and reported this. All PLTs were ordered by a different nurse in charge and responsible for the patient. He/she was not blinded for practical reasons to the treatment arm. A special research nurse collected all data for the study.

Whole blood derived (WBD) buffy coat (BC) PLTs, ABO compatible, leukocyte reduced (<1.0x10 (6) leukocytes/unit), irradiated (25Gy), were prepared according to our earlier standard system (88). Platelet content was 410 ± 20 (mean ± SD) $x10^9$ platelets/unit, suspended in about 70% T-Sol Platelet Additive Solution (Baxter, La Châtre, France) and 30% plasma. The PLTs were stored for five days or less.

3.2.2 Paper IV

Patients ≥ 15 years undergoing allogeneic hematopoetic progenitor cell transplant (HPCT) at Huddinge University Hospital , were randomized to receive PLTs stored for 1-5 days the first time when prophylactic transfusion was needed after transplantation, followed the second time by PLTs stored for 6-7 days or vice versa. Neither the patient nor the ward staff was aware of the storage time of the PLTs transfused.
The corrected count increment (CCI) 1 and 24 hour after transfusion were determined according to the formula; platelet count increment ($x10^9$/L) x body surface area (m^2) / number of platelet transfused ($x10^{11}$/L). Platelet count was analyzed every morning, one hour after transfusion and the following morning (18-24 hours after transfusion). The body surface area was estimated based on the length and weight of the patient and the number of platelet transfused was analyzed.
Nurses from the ward, blinded to the storage time of the PLTs reported if bleeding (i.e.≥ WHO grade 2) (55) was present before the PLT transfusion, the

type of bleeding and whether or not it had stopped within 24 h. Time to the next PLT transfusion within five days after a study PLT was also recorded.

All PLTs were of blood group O, stored up to 7 days, leukoreduced and irradiated as above but prepared according to our present standard OrbiSac (Caridian BCT, Denver, Colorado, USA) system procedure. Details have been reported elsewhere (27). Platelet content was 358 ± 43 x 10 (9)/unit (mean ± SD).

3.3 STATISTICAL ANALYSIS

Mean values and standard deviations (SDs) are usually given. In paper II, III and IV, the statistical comparisons were carried out on a one-to-one basis, with a paired t-test statistic and a two-sided probability of 0.05 to reject the null hypothesis.

In paper I, the Mann-Whitney U-test was used to compare variables expressed by absolute numbers. Differences in distribution between the two groups were compared with the chi-square or Fisher exact test. To calculate the cumulative probability of the outcome variables, the Kaplan-Meier method was used and compared with the log-rank test.

4 RESULTS AND DISCUSSION

4.1 PAPER I

166 patients were included: 79 in group T 10 (transfusion trigger level <10x10^9 platelets/L) and 87 in group T30 (trigger level<30x10^9/L).

No significant differences were found between the two groups as regards patient and donor (of HPC) characteristics or the clinical outcome variables (i.e., bacteremia, engraftment, graft-versus-host disease, hospital stay, death, relapse and survival).

As an indirect measure of bleeding the number of RBC transfusions was recorded. The median total number of red blood cell (RBC) transfusions in the two groups was similar, median 4, range (0-26) group T10, range (0-31) group T30.

However, and in agreement with earlier studies (63, 66-68, 70), the number of PLTs transfused in group T10, using less than 10 x10^9/L as the trigger for prophylactic PLT transfusions, median 4 (0-32), was significantly lower than that in group T30, median 10 (0-48) (P< 0.001). Keeping the patient's platelet count at a higher level required a median of 6 more PLTs. The magnitude of the difference in PLTs use, however, may have been lower if the control arm has had 20 x10^9 platelets/L as trigger level (more common in the United States) instead of 30 x10^9 PLTs/L. The 30 x10^9 platelets/L trigger was hitherto used as the standard in the HPCT unit, based on the routine previously used at the Fred Hutchinson Cancer Research Center in Seattle in 1979, when the program of this unit started.

Median number of days to last PLT transfusion was also reduced in the lower trigger group, 13 (0-30) vs 18 (0-30) (p= 0.002) which is expected since it takes more days to reach a level above 30 than 10 x10^9/L. Another possible explanation might be that frequent PLT transfusions depress the recipient's thrombopoiesis and thus prolong the transfusion period (80).

Beside the trigger level, the number of PLT transfusions and the number of days to the last PLT transfusion were also depended on the day of engraftment and whether acute GVHD or bacteremia were present. PBSC versus bone marrow (BM) also affected the number of days to the last PLT transfusion. This accords with studies comparing the outcome using BM and PBSC grafts, in which the latter grafts were found to have a faster engraftment of neutrophils and platelets (89-92).

The incidence of bleeding (WHO grades 2-4), in T10 18% (14/79), and in T30, 15% (13/87) as well as the type of bleeding were comparable (Table 1).

Table 1. Number and type of hemorrhages

	Group T10	***Group T30***
Epistaxis	7	4
Gastrointestinal	2	4
Gastrointestinal+epistaxis	1	0
Urinary tract	3	3
Cerebral	0	1
Throat and mouth	1	0
Hemorrhage after liver biopsy	0	1
Total grade 2 bleeding	11	8
Total grade3-4 bleeding	3	5
Overall total	14/79	13/87

Thirteen percent (10/79) in the T10 group and 14% (12/87) in the T30 group had had RBC-and/or PLT transfusion-dependent hemorrhages.

In T10, the 14 hemorrhagic events occurred at platelet counts of <5 $x10^9$/L to 122 $x10^9$/L. In the higher trigger group, platelet counts ranged from 9 $x10^9$/L to 65 $x10^9$/L when bleeding was recorded. These findings are in agreement with earlier studies suggesting that patient factors such as graft versus-host-disease (GVHD) for instance, may be more important predictors of hemorrhage than the platelet count (93, 94).
If only patients with serious bleeding were considered – i.e. red blood cell (RBC) transfusion-dependent (WHO grades 3-4) - the platelet counts at the time of hemorrhage were 6, 10 and 12 $x10^9$/L in group T10 and 19, 20, 20, 29 and 65 in group T30. No deaths during the study were attributed to hemorrhages and only one cerebral hemorrhage occurred at a platelet count of 23 $x10^9$/L in the T30 group.

Conclusion:
A prophylactic platelet transfusion trigger level of 10 instead of 30 $x10^9$ platelets/L for allogeneic (HPCT) recipients considerably reduced the number of platelet transfusions without increasing the incidence of hemorrhagic events.

4.2 PAPER II

No significant differences were observed between PLTs stored for 1 vs 7 days regarding mean platelet volume (MPV), pH, pCO_2, pO_2, bicarbonate. Lactate increased and lactic dehydrogenase (LDH) increased slightly, while glucose and ATP decreased, but not to a critical level.

The difficulty of predicting the outcome of platelet transfusion in vivo from the results of laboratory testing, in particular using single in vitro parameters (39-44) is well known. Some in vitro criteria are crucial. Swirling should be present, because its absence is highly predictive of poor posttransfusion platelet count increments; pH should be in the range of 6.4–7.4, and glucose should be present in the PLT throughout storage as energy fuel (7, 10, 41, 45, 46). PLT ATP levels should be >4.0 μmol/10^{11} platelets since lower concentrations may be associated with loss of in vivo viability (47). The results for all of these parameters for PLTs stored for 7 days were acceptable.

The in vitro evaluation of PLT storage lesions also requires a panel of assays able to measure PLT activation and function (48). Hypotonic shock response has been shown to be a suitable in vitro test to predict posttransfusion efficacy (49, 50). In the present study, HSR did not change significantly during storage. RANTES and PF4 are stored in platelet α-granule and released into the PLTs when platelets are activated (41). We found increased concentrations of RANTES (110.7±76.6 vs. 277.6±50.8 pg/10^6 platelets ($p<0.005$)) and PF4 (19.9±9.6 vs. 59.8±7.5 IU/10^6 platelets ($p<0.0001$)) on day 7 compared with day 1. However, the relationship between platelet activation in PLTs during storage and platelet function after transfusion and the effect with regard to posttransfusion recovery and survival in recipients is controversial and needs to be studied in greater detail (44, 51, 52, 95-98).

As regards the in vivo evaluation, platelet recovery was 69±12% on day 1 and 53±13% on day 7, and the survival time 8.2±1.7 days and 5.1±1.7 days, respectively. These values for platelet recovery and survival of fresh and stored PLTs are similar to those reported in previous studies. Mean platelet recovery for fresh PLTs in different studies varied from 55±10% to 87±21% and survival time varied from 5.5±1.5 days to 8.8±0.9 days 31. For 7-day-old PLTs, recovery varied from 36±11% to 64±14 %, and survival time from 4.5±1.6 days to 8.0±0.8 days (31). Most of those studies were from the 1980s with PLTs prepared from platelet rich plasma. However, Dumont et al have studied apheresis PLTs prepared and stored in the same way as in the present study with the exception of the storage medium. Dumont et al used plasma while we used 40% plasma and 60% T-Sol. They found the same recovery (53.9±4.4% vs. 53±13%) and a survival time 161±8.1 hr vs. 5.1±1.7 days) as we did (30).
Another recent study comparing viability and function of 8 day stored apheresis PLTs in plasma, using the newly established guidelines for radiolabeling and how to best compare fresh and stored PLTs (FDA, Workshop on use of radiolabeled platelets for assessment of in vivo viability of platelet products Rockville (MD), 2004), showed recovery of 53 ±20 % and a survival time of 5.6±1.6 days (99).
The data presented in this study show, as expected, a decline in in vivo characteristics after storage of 7 days. There are, however, no officially recognized limits for acceptable results of autologous radionuclide labeling studies**.** Scott Murphy has proposed that mean recovery after storage of platelets should be >67% of that of fresh platelets, and mean cell life should be greater than half of that of fresh platelets (54).

Our results, with platelet recovery on day 7 being 80% of that on day 1, and survival time on day 7 being 65% of that of day 1 (Table 2), meet Murphy's demand.

Table 2.
Platelet recovery and survival time of autologous PLTs after storage for 1 and 7 days.

	Recovery	Survival
Day 1	69±12 (%)	8.2±1.7 (days)
Day 7	53±13 (%)	5.1±1.7 (days)
p-value	<0.05	<0.005
Day 7 as compared with day 1	80±32 (%)	65±26 (%)

Conclusion:
Analyzed in vitro parameters showed acceptable results.
Recovery and survival of PLTs stored for 7 days decreased, but met suggested criteria.

4.3 PAPER III

In vitro parameters for SSP+ (MacoPharma, containing potassium and magnesium) showed significantly reduced glycolysis (lower glucose consumption and decreased production of lactate), a higher HSR and ESC reactivity and a lower degree of platelet activation by means of RANTES, CD62p (P-selectin) and CD63 (proposed marker for the lysosomal granule release (100)) expression as compared to Intersol (Baxter, without these additives). These results are in line with earlier studies (24, 101, 102). It has been proposed that magnesium and citrate modify potassium efflux through platelet membranes and that magnesium inhibits exposure of α-granule glycoprotein GMP-140, decreases the ADP-induced binding of fibrinogen and platelet aggregation (103, 104).
P-selectin (CD62p), a membrane protein released from α granule onto the cell surface of the platelet upon activation, is frequently used as a marker of platelet activation, yet its clinical relevance is uncertain. It has been suggested that increased rate of clearance of platelets from the circulation is correlated to increased expression of P-selectin but that loss of in vivo viability first occurs with relatively high levels of platelets positive for P-selectin (above 50-60%) (41). Michelson et al also 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 (52).

Survival results after 7 days storage showed no significant difference between the two storage solutions 4.2±1.9 days (Intersol) vs 3.6±1.4 days (SSP+). In contrast, recovery for Intersol was significantly higher compared to SSP+, 65± 11% vs 53± 13% (p=0.023) but distribution was wide and the relevance of this finding is uncertain.
There is great variation in results among normal donors regarding platelet recovery and survival. The recovery results after 7 days storage of both PAS in the present study are in line with earlier results discussed above (paper II) but survival was shorter in both storage solutions maybe due to individual differences in the study population. Although limited data are available on the effects of storage time on posttransfusion recovery in patients, there may be reason to believe that the shorter survival time found in healthy subjects may not be as important in patients because of the rapid consumption of platelets in thrombocytopenic patients due to the fact that platelet viability also depends on the severity of thrombocytopenia, the underlying illness and the presence of HLA and ABO antibodies. Several studies have demonstrated the impact of such patient factors on the occurrence of transfusion failure (58, 105-110).
In our previous studies the in vivo results were very conclusive. In those studies we used only one radionuclide (^{111}Indium) (87, 111). In the present study we used two different radionuclide for the first time and the results are inconclusive in comparison with in vitro data. Despite the validation study of extended storage with PLTs stored in plasma performed by AuBuchon et al (29), to determine whether ^{111}Indium and ^{51}Chromium radiolabel could be used interchangeably and where no difference in either recovery or survival according to the radiolabel used was found. However, other authors (99) have reported problems with the use of two radionuclides despite having applied the newly established guidelines for radiolabeling with two radionuclides (FDA, Workshop on use of radiolabeled platelets for assessment of in vivo viability of platelet products Rockville (MD), 2004.). As Slichter et al noticed in their study, we also experienced higher PLT recoveries with indium than with chromium label (99). On the other hand, the recovery was higher with Intersol than with SSP+ in 8 of 10 donors involved in our study. In agreement with Slichters data we also noticed that PLT survivals tended to be shorter with indium labeling (99). Unfortunately, we have no data on the effects of labeling platelets with either indium or chromium using the same storage environment with our labeling technique and further studies will be needed.

Conclusion
In vitro characteristics of PLTs stored in PAS with addition of potassium and magnesium indicated higher quality but this could not be verified by the in vivo parameters by means of recovery and survival.

4.4 PAPER IV

60 allogeneic HPCT recipients were evaluated. Mean storage time for 1-5 days PLTs was 2.9 ±1.1 days (median 3 days) and 6.6 ± 0.5 days (median 7 days) for the 6-7 group.

The CCI 1 hour and CCI 24 hour after transfusion were higher for PLTs stored 1-5 days as compared to PLTs stored 6-7 days, 10.4 ± 5.1 vs 7.4 ± 3.8 ($p< 0.001$) and 5.4 ± 4.1 vs 2.6 ± 2.6 ($p< 0.001$) respectively. (Table 3)

When PLTs are transfused into a patient who shows signs of active bleeding at the time of transfusion, the bleeding usually stops, even when there is no apparent increase in the platelet count and the PLTs transfused have been stored for several days (112). Therefore the haemostatic effects of stored PLTs is not usually questioned. On the other hand, most patients receive prophylactic platelet transfusions (59) and under these circumstances, a successful transfusion is usually defined as an increase in the peripheral platelet count. Consequently, the posttransfusion platelet increment or corrected count increment (CCI) is still the golden standard for posttransfusion platelet survival.

Studies examining CCI in thrombocytopenic patients are difficult to compare since both the selected patient groups and the methods used to prepare the platelet products varies substantially (31, 32), although a CCI 1 hour and 24 hour of 7.5 and 4.5 respectively is generally considered to be a successful increment (73). Consequently, the mean CCI of the older PLTs in this study did not reach the level considered as a successful increment (Table 3). But, as already has been pointed out, it is important to take into account that poor CCI values are not necessarily due to poor product viability alone but may be due to the specific condition in different patient categories and to what extent it interacts with the storage lesion in PLTs older than 5 days. The poor response to platelet transfusions in allogeneic HPCT recipients is well known (105-108, 113) but the underlying pathophysiologic mechanisms has not yet been identified although endothelial vascular damage caused by cytotoxic treatment or disease-related complications has been proposed potential mechanisms (94, 107, 110).

In general, extension of platelet storage time from 5 to 7 days results in small but statistically significant decline in the posttransfusion recovery and survival.(29, 31, 32) The difference has been deemed to be clinically irrelevant. In most cases of a prophylactic transfusion, the decision of transfusion is made on the platelet count taken in the morning and depending on a predetermined threshold. Small differences in platelet increment may therefore have a considerable effect on the number of platelet transfusions as has been indicated by a mathematical model of prophylactic platelet support (114).

As been shown by others, the CCI for prophylactic platelet transfusions correlates with the transfusion interval, lower CCI results in shorter interval and vice versa (106, 110). In our study the lower CCI for PLTs stored for 6-7 days gave a transfusion interval of 1.6 ± 0.8 days (median 1.5) as compared to 2.2 ± 1.1 days (median 3.5) for platelets stored for 1-5days ($p<0.005$) (Table 3). Longer intertransfusion interval represents a considerable difference in quality of life for outpatients and less workload for the hospital staff.

Table 3.
Results of CCI 1 and 24 hour and transfusion interval, expressed as means ± 1 SD.

	PLTs 1-5 days	PLTs 6-7 days	
CCI 1h n=60	10.4 ± 5.1	7.4 ± 3.8	p< 0.001
CC1 24h n=58	5.4 ± 4.1	2.6 ± 2.6	p< 0.001
PLT Intertransfusion interval (days) n=40	2.2 ± 1.1 (median 3.5)	1.6 ± 0.8 (median 1.5)	p< 0.005

Bleeding episodes were not a primary endpoint in this study. This is due to several reasons including the difficulty of bleeding assessment and grading (57). In addition, the HPCT recipients given prophylactic platelet transfusion, hemorrhages is frequently due to other reasons than thrombocytopenia, reflecting the ongoing complications of HPC transplantation such as mucositis, hemorrhagic cystitis, GVHD, VOD and diffuse alveolar damage (94, 115).

A recent study in HPCT recipients without clinically significant bleeding has shown an association of profound thrombocytopenia with reduced patient survival and a relation with increased platelet consumption due to endothelial damage has been suggested (116).

Nevertheless, a total of 25 hemorrhages with ≥ WHO grade 2 were reported in this study. No hemorrhage was graded WHO 4. Epistaxis was most frequent, 20/25. The remaining five cases consisted of gastrointestinal bleeding and hemorrhagic cystitis where bleeding was still present 24 hrs after platelet transfusion, regardless of the PLTs transfused (Table 4).

For the patients with epistaxis who received PLTs stored for 1-5 day, bleeding stopped within 24 hrs in 4/7. The corresponding data for patients who received PLTs stored for 6-7 day was 8/13 (Table 4).

Table 4.
Incidence and type of hemorrhage (≥ WHO grade 2) recorded before PLT transfusion. Continued i.e. bleeding still present 24 h after transfusion.

	PLTs 1-5 days		PLTs 6-7 days	
Bleeding	stopped	continued	stopped	continued
Epistaxis n=20	4	3	8	5
Gastrointestinal and/or hemorrhagic cystitis n=5	0	2	0	3

Conclusion
Prophylactic transfusion in allogeneic HPCT recipients with BC PLTs stored in PAS for 6-7 days resulted in lower CCI and shorter interval between transfusions. Therefore the advantage of an extension of platelet storage time beyond day 5 should be balanced against the increased need for platelet transfusions that may occur and the conceivable risk of transfusion failure.

5 CONCLUDING REMARKS

An important aim in transfusion medicine is to ensure an adequate supply of PLTs of optimal quality for transfusion. Due to the uneven demand and the short storage period of PLTs, there are sometimes PLT shortages, especially during extended holiday periods. Provided that contaminating bacteria can be effectively detected or put out of action in PLTs, prolonged storage represents one possibility to obtain improved availability, logistical management and decreased outdating (25). Another alternative is to cut down the prophylactic platelet transfusions whenever possible.

There may be several advantages in reducing the number of prophylactic platelet transfusions.
1. Reduction of PLT shortages. In view of the steady increase in requests for PLTs from blood banks, prophylactic platelet administration must be restricted to ensure an adequate supply when really needed. About 2 million PLTs are transfused per year in the United States (60), 2.9 million in Europe (12) and 40 000 in Sweden with a roughly ten-fold increase since 1984 and an increase by >8 % 2007 as compared to 2006 (Transfusion services in Sweden 2007: extent, quality and safety. Swedish Society for Transfusion Medicine). Continued increase in demand may result in difficulties to comply with orders.
2. Avoidance of undesirable side effects. These include fever, shivering, allergic reactions, septicemia due to bacterial contamination, viral transmission, immunization with subsequent PLT refractoriness, etc., which may be associated with platelet transfusions (51, 117-129).
3. Cost savings. PLT transfusions are expensive.

Early studies in the 1960s showed that PLT transfusions significantly reduced the incidence of fatal hemorrhages (2, 3). Since then there has been a change from therapeutic -i.e., treatment of clinical bleeding- to prophylactic platelet transfusions. In a 1991 survey in the US, more than 70% of hospitals reported transfusing platelets primarily for prophylaxis (59) despite limited clinical evidence regarding whether a prophylactic platelet transfusion strategy results in a lower morbidity or mortality compared with transfusing patients with early signs of bleeding. When platelet transfusions became established practice, it took several hours or even a day before the request could be complied with. Today, when PLTs (hopefully) are kept in stock, the need of prophylactic transfusions needs to be revised with respect to different patient categories.

In paper I we showed that a prophylactic platelet transfusion trigger level of 10 instead of 30 $x10^9$ platelets/L for HPCT recipients considerably reduced the number of platelet transfusions without increasing the incidence of hemorrhagic events.
However, lately there have been suggestions that HPCT recipients might not be a patient category to study with only hemorrhages as an outcome measure when lowering trigger to decrease the demand of prophylactic transfusions.
Firstly, in HPCT recipients given prophylactic platelet transfusion, hemorrhages is frequently due to other reasons than thrombocytopenia, reflecting the ongoing

complications of HPC transplantation such as mucositis, hemorrhagic cystitis, GVHD, VOD and diffuse alveolar damage (94, 115)
Secondly, in a recently published retrospective study it has been shown that a trigger of 10 instead of 20 x 10^9 platelets/L was associated with significantly greater exposure to lower platelet count. Further, nonbleeding patients with profound thrombocytopenia were at significantly greater risk of dying compared with nonthrombocytopenic patients and a relation with increased platelet consumption due to endothelial damage has been suggested. Therefore, patient survival has been proposed to be a more clinically relevant outcome for platelet transfusions studies in this patient category (116). However, assigning a cause for mortality in patients with hematological malignancies is complicated.
If thrombocytopenia is just a surrogate marker then the trigger may be irrelevant; but if thrombocytopenia accelerates clinical deterioration perhaps more vigorous platelet support and a higher platelet transfusion trigger might improve the clinical outcome in thrombocytopenic nonbleeding patients (116).

Although no significant differences were found between the two groups in our study as regards the outcome of the transplantation during the three-year follow up –i.e. the transplantation-related mortality, survival and disease-free survival, the study was not designed for that issue.

A different approach to affect the accessibility of PLTs is to prolong storage. While this option may appear to be a solution for improving platelet availability, it can also lead to increased testing and handling costs associated with increased bacteria testing or pathogen inactivation and lower platelet quality, thereby reducing transfusion intervals and increasing overall demand and cost for platelets. In paper II, recovery and survival in healthy volunteers of PLTs stored for 7 days decreased, but met the suggested criteria. Analyzed in vitro parameters showed acceptable results. However when comparing prophylactic transfusion in allogeneic HPCT recipients of BC PLTs in PAS stored for 6-7 days vs 1-5 days (paper IV) there was a significant lower CCI and shorter interval between transfusions with prolonged storage.

To have sufficient PLTs of excellent quality in stock at any given time to provide to patients in great need represents a difficult challenge to transfusion services. If prolonged storage results in decreased time between the transfusion and more PLTs are transfused in general –the result might not be what was aimed at. In that case it might be wiser to question the need of prophylactic transfusion. Lately it has been proposed that patient selection may be the key to the safety of therapeutic only based platelet transfusion strategy.

To conclude, there is a growing need of further randomized, controlled clinical trials to establish the therapeutic approaches that maximizes the quality of patient care in different patient categories while minimizing expenses to transfusion services and the health care system to be able to ensure an adequate supply when really needed.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all those who have helped and supported me during the production of this thesis, but above all the following:

Agneta Shanwell, my supervisor, for sharing your profound knowledge in transfusion medicine, for teaching me how to write scientific papers and always being supportive and prepared to share your time.

Hans Gulliksson my co-supervisor, for advice and assistance and for sharing your deep scientific knowledge in the blood component field and also helping me running the blood donation unit during several months, I will always remember.

Joachim Lundahl, head of the Dept of Clinical Immunology and Transfusion Medicine for approval of my research and for providing time off to complete this thesis and also for being a kind-hearted person.

Berit Jansson and Leif Svensson for skilful technical assistance and help with the radionuclide analysis.

My co-authors, Mats Remberger, Olle Ringdén, Britt-Marie Svahn, Emma Watz for good co-operation, and particularly Per Sandgren for all the flow cytometry work and for being so helpful with all kinds of advice.

Bo Nilsson for expert assistance with statistical analysis of data.

All nice and always helpful colleagues.

The whole staff at the Department of Immunology and Transfusion medicine for all kinds of help.

Taina Dekhil, Lottie Furugård och Monica Åqvist for being such strong women.

All my friends since way back and the whole clan Aspevall/Diedrich/Gidenstam, I always think of what a big family we are.

And finally my nearest, my husband and my children for your love and for putting up with me during the preparation of this thesis.

REFERENCES

1. Duke W. The relation of blood platelets to hemorrhagic disease. JAMA. 1910(55):1185-92.
2. Han T, Stutzman L, Cohen E, Kim U. Effect of platelet transfusion on hemorrhage in patients with acute leukemia. An autopsy study. Cancer. 1966 Dec;19(12):1937-42.
3. Hersh EM, Bodey GP, Nies BA, Freireich EJ. Causes of Death in Acute Leukemia: a Ten-Year Study of 414 Patients from 1954-1963. Jama. 1965 Jul 12;193:105-9.
4. Murphy S. Platelet storage for transfusion. Seminars in hematology. 1985 Jul;22(3):165-77.
5. Murphy S, Gardner FH. Platelet storage at 22 degrees C: role of gas transport across plastic containers in maintenance of viability. Blood. 1975 Aug;46(2):209-18.
6. Murphy S, Sayar SN, Gardner FH. Storage of platelet concentrates at 22 degrees C. Blood. 1970 Apr;35(4):549-57.
7. Gulliksson H. Defining the optimal storage conditions for the long-term storage of platelets. Transfusion medicine reviews. 2003 Jul;17(3):209-15.
8. van der Meer PF, Pietersz RN, Reesink HW. Storage of platelets in additive solution for up to 12 days with maintenance of good in-vitro quality. Transfusion. 2004 Aug;44(8):1204-11.
9. Blajchman MA, Ali AM, Richardson HL. Bacterial contamination of cellular blood components. Vox sanguinis. 1994;67 Suppl 3:25-33.
10. Publishing CoE. Guide to the preparation, use and quality assurance of blood components, 13 th edition. 13th ed 2007.
11. Brecher ME, Hay SN. Bacterial contamination of blood components. Clinical microbiology reviews. 2005 Jan;18(1):195-204.
12. Stroncek DF, Rebulla P. Platelet transfusions. Lancet. 2007 Aug 4;370(9585):427-38.
13. Allain JP, Bianco C, Blajchman MA, Brecher ME, Busch M, Leiby D, et al. Protecting the blood supply from emerging pathogens: the role of pathogen inactivation. Transfusion medicine reviews. 2005 Apr;19(2):110-26.
14. McCullough J, Vesole DH, Benjamin RJ, Slichter SJ, Pineda A, Snyder E, et al. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. Blood. 2004 Sep 1;104(5):1534-41.
15. Murphy S, Snyder E, Cable R, Slichter SJ, Strauss RG, McCullough J, et al. Platelet dose consistency and its effect on the number of platelet transfusions for support of thrombocytopenia: an analysis of the SPRINT trial of platelets photochemically treated with amotosalen HCl and ultraviolet A light. Transfusion. 2006 Jan;46(1):24-33.
16. Simonsen AC, Johansson PI, Conlan MG, Jacquet M, Lin JS, Junge K, et al. Transfusion of 7-day-old amotosalen photochemically treated buffy-coat platelets to patients with thrombocytopenia: a pilot study. Transfusion. 2006 Mar;46(3):424-33.
17. Slichter SJ, Raife TJ, Davis K, Rheinschmidt M, Buchholz DH, Corash L, et al. Platelets photochemically treated with amotosalen HCl and ultraviolet A light correct prolonged bleeding times in patients with thrombocytopenia. Transfusion. 2006 May;46(5):731-40.
18. Snyder E, Raife T, Lin L, Cimino G, Metzel P, Rheinschmidt M, et al. Recovery and life span of 111indium-radiolabeled platelets treated with pathogen inactivation with amotosalen HCl (S-59) and ultraviolet A light. Transfusion. 2004 Dec;44(12):1732-40.
19. van Rhenen D, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Kluter H, et al. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood. 2003 Mar 15;101(6):2426-33.
20. Vassallo RR, Murphy S. A critical comparison of platelet preparation methods. Current opinion in hematology. 2006 Sep;13(5):323-30.
21. Pietersz RN, Reesink HW, Dekker WJ, Fijen FJ. Preparation of leukocyte-poor platelet concentrates from buffy coats. I. Special inserts for centrifuge cups. Vox sanguinis. 1987;53(4):203-7.

22. Burgstaler EA. Blood component collection by apheresis. Journal of clinical apheresis. 2006 Jul;21(2):142-51.
23. Heddle NM, Klama L, Singer J, Richards C, Fedak P, Walker I, et al. The role of the plasma from platelet concentrates in transfusion reactions. The New England journal of medicine. 1994 Sep 8;331(10):625-8.
24. Gulliksson H, AuBuchon JP, Cardigan R, van der Meer PF, Murphy S, Prowse C, et al. Storage of platelets in additive solutions: a multicentre study of the in vitro effects of potassium and magnesium. Vox sanguinis. 2003 Oct;85(3):199-205.
25. Hay SN, Immel CC, McClannan LS, Brecher ME. The introduction of 7-day platelets: a university hospital experience. Journal of clinical apheresis. 2007;22(5):283-6.
26. Dumont LJ, VandenBroeke T. Seven-day storage of apheresis platelets: report of an in vitro study. Transfusion. 2003 Feb;43(2):143-50.
27. Larsson S, Sandgren P, Sjodin A, Vesterinen M, Gulliksson H. Automated preparation of platelet concentrates from pooled buffy coats: in vitro studies and experiences with the OrbiSac system. Transfusion. 2005 May;45(5):743-51.
28. Murphy S. The efficacy of synthetic media in the storage of human platelets for transfusion. Transfusion medicine reviews. 1999 Jul;13(3):153-63.
29. AuBuchon JP, Taylor H, Holme S, Nelson E. In vitro and in vivo evaluation of leukoreduced platelets stored for 7 days in CLX containers. Transfusion. 2005 Aug;45(8):1356-61.
30. Dumont LJ, AuBuchon JP, Whitley P, Herschel LH, Johnson A, McNeil D, et al. Seven-day storage of single-donor platelets: recovery and survival in an autologous transfusion study. Transfusion. 2002 Jul;42(7):847-54.
31. Cardigan R, Williamson LM. The quality of platelets after storage for 7 days. Transfusion medicine (Oxford, England). 2003 Aug;13(4):173-87.
32. Dijkstra-Tiekstra MJ, Pietersz RN, Hendriks EC, Reesink HW, Huijgens PC. In vivo PLT increments after transfusions of WBC-reduced PLT concentrates stored for up to 7 days. Transfusion. 2004 Mar;44(3):330-6.
33. Kilkson H, Holme S, Murphy S. Platelet metabolism during storage of platelet concentrates at 22 degrees C. Blood. 1984 Aug;64(2):406-14.
34. Guppy M, Whisson ME, Sabaratnam R, Withers P, Brand K. Alternative fuels for platelet storage: a metabolic study. Vox sanguinis. 1990;59(3):146-52.
35. Gulliksson H, Eriksson L, Hogman CF, Payrat JM. Buffy-coat-derived platelet concentrates prepared from half-strength citrate CPD and CPD whole-blood units. Comparison between three additive solutions: in vitro studies. Vox sanguinis. 1995;68(3):152-9.
36. van der Meer PF, Gulliksson H, Aubuchon JP, Prowse C, Richter E, de Wildt-Eggen J. Interruption of agitation of platelet concentrates: effects on in vitro parameters. Vox sanguinis. 2005 May;88(4):227-34.
37. Murphy S, Gardner FH. Effect of storage temperature on maintenance of platelet viability--deleterious effect of refrigerated storage. The New England journal of medicine. 1969 May 15;280(20):1094-8.
38. Seghatchian J, Krailadsiri P. The platelet storage lesion. Transfusion medicine reviews. 1997 Apr;11(2):130-44.
39. Cardigan R, Turner C, Harrison P. Current methods of assessing platelet function: relevance to transfusion medicine. Vox sanguinis. 2005 Apr;88(3):153-63.
40. Evaluation of stored platelets. Vox sanguinis. 2004 Apr;86(3):203-23.
41. Holme S. Storage and quality assessment of platelets. Vox sanguinis. 1998;74 Suppl 2:207-16.
42. Murphy S. Utility of in vitro tests in predicting the in vivo viability of stored PLTs. Transfusion. 2004 Apr;44(4):618-9; author reply 9.
43. Murphy S, Rebulla P, Bertolini F, Holme S, Moroff G, Snyder E, et al. In vitro assessment of the quality of stored platelet concentrates. The BEST (Biomedical Excellence for Safer Transfusion) Task Force of the International Society of Blood Transfusion. Transfusion medicine reviews. 1994 Jan;8(1):29-36.
44. Rinder HM, Smith BR. In vitro evaluation of stored platelets: is there hope for predicting posttransfusion platelet survival and function? Transfusion. 2003 Jan;43(1):2-6.

45. Bertolini F, Agazzi A, Peccatori F, Martinelli G, Sandri MT. The absence of swirling in platelet concentrates is highly predictive of poor posttransfusion platelet count increments and increased risk of a transfusion reaction. Transfusion. 2000 Jan;40(1):121-2.
46. Gulliksson H. Additive solutions for the storage of platelets for transfusion. Transfusion medicine (Oxford, England). 2000 Dec;10(4):257-64.
47. Holme S, Heaton WA, Courtright M. Platelet storage lesion in second-generation containers: correlation with platelet ATP levels. Vox sanguinis. 1987;53(4):214-20.
48. Snyder EL. Activation during preparation and storage of platelet concentrates. Transfusion. 1992 Jul-Aug;32(6):500-2.
49. Holme S, Moroff G, Murphy S. A multi-laboratory evaluation of in vitro platelet assays: the tests for extent of shape change and response to hypotonic shock. Biomedical Excellence for Safer Transfusion Working Party of the International Society of Blood Transfusion. Transfusion. 1998 Jan;38(1):31-40.
50. Valeri CR, Feingold H, Marchionni LD. The relation between response to hypotonic stress and the 51Cr recovery in vivo of preserved platelets. Transfusion. 1974 Jul-Aug;14(4):331-7.
51. Kluter H, Bubel S, Kirchner H, Wilhelm D. Febrile and allergic transfusion reactions after the transfusion of white cell-poor platelet preparations. Transfusion. 1999 Nov-Dec;39(11-12):1179-84.
52. Michelson AD, Barnard MR, Hechtman HB, MacGregor H, Connolly RJ, Loscalzo J, et al. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. Proceedings of the National Academy of Sciences of the United States of America. 1996 Oct 15;93(21):11877-82.
53. Murphy S, Holme S, Nelson E, Carmen R. Paired comparison of the in vivo and in vitro results of storage of platelet concentrates in two containers. Transfusion. 1984 Jan-Feb;24(1):31-4.
54. Murphy S. Radiolabeling of PLTs to assess viability: a proposal for a standard. Transfusion. 2004 Jan;44(1):131-3.
55. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer. 1981 Jan 1;47(1):207-14.
56. Slichter SJ. Platelet transfusion therapy. Hematology/oncology clinics of North America. 2007 Aug;21(4):697-729, vii.
57. Cook RJ, Heddle NM, Rebulla P, Sigouin CS, Webert KE. Methods for the analysis of bleeding outcomes in randomized trials of PLT transfusion triggers. Transfusion. 2004 Aug;44(8):1135-42.
58. Hanson SR, Slichter SJ. Platelet kinetics in patients with bone marrow hypoplasia: evidence for a fixed platelet requirement. Blood. 1985 Nov;66(5):1105-9.
59. Pisciotto PT, Benson K, Hume H, Glassman AB, Oberman H, Popovsky M, et al. Prophylactic versus therapeutic platelet transfusion practices in hematology and/or oncology patients. Transfusion. 1995 Jun;35(6):498-502.
60. Heal JM, Blumberg N. Optimizing platelet transfusion therapy. Blood reviews. 2004 Sep;18(3):149-65.
61. Beutler E. Platelet transfusions: the 20,000/microL trigger. Blood. 1993 Mar 15;81(6):1411-3.
62. Gaydos LA, Freireich EJ, Mantel N. The quantitative relation between platelet count and hemorrhage in patients with acute leukemia. The New England journal of medicine. 1962 May 3;266:905-9.
63. Gil-Fernandez JJ, Alegre A, Fernandez-Villalta MJ, Pinilla I, Gomez Garcia V, Martinez C, et al. Clinical results of a stringent policy on prophylactic platelet transfusion: non-randomized comparative analysis in 190 bone marrow transplant patients from a single institution. Bone marrow transplantation. 1996 Nov;18(5):931-5.
64. Gmur J, Burger J, Schanz U, Fehr J, Schaffner A. Safety of stringent prophylactic platelet transfusion policy for patients with acute leukaemia. Lancet. 1991 Nov 16;338(8777):1223-6.
65. Heckman KD, Weiner GJ, Davis CS, Strauss RG, Jones MP, Burns CP. Randomized study of prophylactic platelet transfusion threshold during induction therapy for adult acute leukemia: 10,000/microL versus 20,000/microL. J Clin Oncol. 1997 Mar;15(3):1143-9.

66. Lawrence JB, Yomtovian RA, Hammons T, Masarik SR, Chongkolwatana V, Creger RJ, et al. Lowering the prophylactic platelet transfusion threshold: a prospective analysis. Leukemia & lymphoma. 2001 Mar;41(1-2):67-76.
67. Navarro JT, Hernandez JA, Ribera JM, Sancho JM, Oriol A, Pujol M, et al. Prophylactic platelet transfusion threshold during therapy for adult acute myeloid leukemia: 10,000/microL versus 20,000/microL. Haematologica. 1998 Nov;83(11):998-1000.
68. Rebulla P, Finazzi G, Marangoni F, Avvisati G, Gugliotta L, Tognoni G, et al. The threshold for prophylactic platelet transfusions in adults with acute myeloid leukemia. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto. The New England journal of medicine. 1997 Dec 25;337(26):1870-5.
69. Sagmeister M, Oec L, Gmur J. A restrictive platelet transfusion policy allowing long-term support of outpatients with severe aplastic anemia. Blood. 1999 May 1;93(9):3124-6.
70. Wandt H, Frank M, Ehninger G, Schneider C, Brack N, Daoud A, et al. Safety and cost effectiveness of a 10 x 10(9)/L trigger for prophylactic platelet transfusions compared with the traditional 20 x 10(9)/L trigger: a prospective comparative trial in 105 patients with acute myeloid leukemia. Blood. 1998 May 15;91(10):3601-6.
71. Zumberg MS, del Rosario ML, Nejame CF, Pollock BH, Garzarella L, Kao KJ, et al. A prospective randomized trial of prophylactic platelet transfusion and bleeding incidence in hematopoietic stem cell transplant recipients: 10,000/L versus 20,000/microL trigger. Biol Blood Marrow Transplant. 2002;8(10):569-76.
72. Stanworth SJ, Hyde C, Heddle N, Rebulla P, Brunskill S, Murphy MF. Prophylactic platelet transfusion for haemorrhage after chemotherapy and stem cell transplantation. Cochrane database of systematic reviews (Online). 2004(4):CD004269.
73. Guidelines for the use of platelet transfusions. British journal of haematology. 2003 Jul;122(1):10-23.
74. Blajchman MA, Slichter SJ, Heddle NM, Murphy MF. New strategies for the optimal use of platelet transfusions. Hematology / the Education Program of the American Society of Hematology American Society of Hematology. 2008;2008:198-204.
75. Slichter SJ. Relationship between platelet count and bleeding risk in thrombocytopenic patients. Transfusion medicine reviews. 2004 Jul;18(3):153-67.
76. Stanworth SJ, Hyde C, Brunskill S, Murphy MF. Platelet transfusion prophylaxis for patients with haematological malignancies: where to now? British journal of haematology. 2005 Dec;131(5):588-95.
77. Friedmann AM, Sengul H, Lehmann H, Schwartz C, Goodman S. Do basic laboratory tests or clinical observations predict bleeding in thrombocytopenic oncology patients? A reevaluation of prophylactic platelet transfusions. Transfusion medicine reviews. 2002 Jan;16(1):34-45.
78. Wandt H, Schaefer-Eckart K, Frank M, Birkmann J, Wilhelm M. A therapeutic platelet transfusion strategy is safe and feasible in patients after autologous peripheral blood stem cell transplantation. Bone marrow transplantation. 2006 Feb;37(4):387-92.
79. Sensebe L, Giraudeau B, Bardiaux L, Deconinck E, Schmidt A, Bidet ML, et al. The efficiency of transfusing high doses of platelets in hematologic patients with thrombocytopenia: results of a prospective, randomized, open, blinded end point (PROBE) study. Blood. 2005 Jan 15;105(2):862-4.
80. Slichter SJ. Background, rationale, and design of a clinical trial to assess the effects of platelet dose on bleeding risk in thrombocytopenic patients. Journal of clinical apheresis. 2006 Apr;21(1):78-84.
81. Heddle NM, Cook RJ, Tinmouth A, Kouroukis CT, Hervig T, Klapper E, et al. A randomized controlled trial comparing standard- and low-dose strategies for transfusion of platelets (SToP) to patients with thrombocytopenia. Blood. 2009 Feb 12;113(7):1564-73.
82. Moroff G, Eich J, Dabay M. Validation of use of the Nageotte hemocytometer to count low levels of white cells in white cell-reduced platelet components. Transfusion. 1994 Jan;34(1):35-8.
83. Lundin A. Use of firefly luciferase in ATP-related assays of biomass, enzymes, and metabolites. Methods in enzymology. 2000;305:346-70.

84. VandenBroeke T, Dumont LJ, Hunter S, Nixon J, Murphy S, Roger J, et al. Platelet storage solution affects on the accuracy of laboratory tests for platelet function: a multi-laboratory study. Vox sanguinis. 2004 Apr;86(3):183-8.
85. Bertolini F, Murphy S. A multicenter evaluation of reproducibility of swirling in platelet concentrates. Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion. Transfusion. 1994 Sep;34(9):796-801.
86. Sandgren P, Hansson M, Gulliksson H, Shanwell A. Storage of buffy-coat-derived platelets in additive solutions at 4 degrees C and 22 degrees C: flow cytometry analysis of platelet glycoprotein expression. Vox sanguinis. 2007 Jul;93(1):27-36.
87. Shanwell A, Gulliksson H, Berg BK, Jansson BA, Svensson LA. Evaluation of platelets prepared by apheresis and stored for 5 days. In vitro and in vivo studies. Transfusion. 1989 Nov-Dec;29(9):783-8.
88. Gulliksson H, Larsson S, Shanwell A, Pedajas I, Joie M, Flament J. A system for the supply of platelets suspended in a storage medium including buffy-coat-derived platelet concentrates in combination with "split" apheresis platelets. Transfusion science. 1997 Mar;18(1):33-40.
89. Bensinger WI, Clift R, Martin P, Appelbaum FR, Demirer T, Gooley T, et al. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: a retrospective comparison with marrow transplantation. Blood. 1996 Oct 1;88(7):2794-800.
90. Bensinger WI, Weaver CH, Appelbaum FR, Rowley S, Demirer T, Sanders J, et al. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. Blood. 1995 Mar 15;85(6):1655-8.
91. Korbling M, Przepiorka D, Huh YO, Engel H, van Besien K, Giralt S, et al. Allogeneic blood stem cell transplantation for refractory leukemia and lymphoma: potential advantage of blood over marrow allografts. Blood. 1995 Mar 15;85(6):1659-65.
92. Ringden O, Labopin M, Bacigalupo A, Arcese W, Schaefer UW, Willemze R, et al. Transplantation of peripheral blood stem cells as compared with bone marrow from HLA-identical siblings in adult patients with acute myeloid leukemia and acute lymphoblastic leukemia. J Clin Oncol. 2002 Dec 15;20(24):4655-64.
93. Bernstein SH, Nademanee AP, Vose JM, Tricot G, Fay JW, Negrin RS, et al. A multicenter study of platelet recovery and utilization in patients after myeloablative therapy and hematopoietic stem cell transplantation. Blood. 1998 May 1;91(9):3509-17.
94. Nevo S, Enger C, Swan V, Wojno KJ, Fuller AK, Altomonte V, et al. Acute bleeding after allogeneic bone marrow transplantation: association with graft versus host disease and effect on survival. Transplantation. 1999 Mar 15;67(5):681-9.
95. Berger G, Hartwell DW, Wagner DD. P-Selectin and platelet clearance. Blood. 1998 Dec 1;92(11):4446-52.
96. Holme S, Sweeney JD, Sawyer S, Elfath MD. The expression of p-selectin during collection, processing, and storage of platelet concentrates: relationship to loss of in vivo viability. Transfusion. 1997 Jan;37(1):12-7.
97. Rinder HM, Murphy M, Mitchell JG, Stocks J, Ault KA, Hillman RS. Progressive platelet activation with storage: evidence for shortened survival of activated platelets after transfusion. Transfusion. 1991 Jun;31(5):409-14.
98. Wakamoto S, Fujihara M, Kuzuma K, Sato S, Kato T, Naohara T, et al. Biologic activity of RANTES in apheresis PLT concentrates and its involvement in nonhemolytic transfusion reactions. Transfusion. 2003 Aug;43(8):1038-46.
99. Slichter SJ, Bolgiano D, Jones MK, Christoffel T, Corson J, Rose L, et al. Viability and function of 8-day-stored apheresis platelets. Transfusion. 2006 Oct;46(10):1763-9.
100. Nieuwenhuis HK, van Oosterhout JJ, Rozemuller E, van Iwaarden F, Sixma JJ. Studies with a monoclonal antibody against activated platelets: evidence that a secreted 53,000-molecular weight lysosome-like granule protein is exposed on the surface of activated platelets in the circulation. Blood. 1987 Sep;70(3):838-45.
101. de Wildt-Eggen J, Schrijver JG, Bins M, Gulliksson H. Storage of platelets in additive solutions: effects of magnesium and/or potassium. Transfusion. 2002 Jan;42(1):76-80.

102. Shanwell A, Falker C, Gulliksson H. Storage of platelets in additive solutions: the effects of magnesium and potassium on the release of RANTES, beta-thromboglobulin, platelet factor 4 and interleukin-7, during storage. Vox sanguinis. 2003 Oct;85(3):206-12.
103. Gawaz M, Ott I, Reininger AJ, Neumann FJ. Effects of magnesium on platelet aggregation and adhesion. Magnesium modulates surface expression of glycoproteins on platelets in vitro and ex vivo. Thrombosis and haemostasis. 1994 Dec;72(6):912-8.
104. Weis-Fogh US. The effect of citrate, calcium, and magnesium ions on the potassium movement across the human platelet membrane. Transfusion. 1985 Jul-Aug;25(4):339-42.
105. Bishop JF, McGrath K, Wolf MM, Matthews JP, De Luise T, Holdsworth R, et al. Clinical factors influencing the efficacy of pooled platelet transfusions. Blood. 1988 Feb;71(2):383-7.
106. Heim D, Passweg J, Gregor M, Buser A, Theocharides A, Arber C, et al. Patient and product factors affecting platelet transfusion results. Transfusion. 2008 Apr;48(4):681-7.
107. Ishida A, Handa M, Wakui M, Okamoto S, Kamakura M, Ikeda Y. Clinical factors influencing posttransfusion platelet increment in patients undergoing hematopoietic progenitor cell transplantation--a prospective analysis. Transfusion. 1998 Sep;38(9):839-47.
108. Klumpp TR, Herman JH, Innis S, Pearlman E, Culling N, Kotz KW, et al. Factors associated with response to platelet transfusion following hematopoietic stem cell transplantation. Bone marrow transplantation. 1996 Jun;17(6):1035-41.
109. Norol F, Kuentz M, Cordonnier C, Beaujean F, Haioun C, Vernant JP, et al. Influence of clinical status on the efficiency of stored platelet transfusion. British journal of haematology. 1994 Jan;86(1):125-9.
110. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao KJ, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood. 2005 May 15;105(10):4106-14.
111. Shanwell A, Diedrich B, Falker C, Jansson B, Sandgren P, Sundkvist L, et al. Paired in vitro and in vivo comparison of apheresis platelet concentrates stored in platelet additive solution for 1 versus 7 days. Transfusion. 2006 Jun;46(6):973-9.
112. Taylor MA, Tandy NP, Fraser ID. Effect of new plastics and leucocyte contamination on in vitro storage of platelet concentrates. Journal of clinical pathology. 1983 Dec;36(12):1382-6.
113. Shanwell A, Larsson S, Aschan J, Ringden O. A randomized trial comparing the use of fresh and stored platelets in the treatment of bone marrow transplant recipients. European journal of haematology. 1992 Aug;49(2):77-81.
114. Pereira A, Sanz C. Effect of extending the platelet storage time on platelet utilization: predictions from a mathematical model of prophylactic platelet support. Transfusion medicine (Oxford, England). 2007 Apr;17(2):119-27.
115. Nevo S, Fuller AK, Hartley E, Borinsky ME, Vogelsang GB. Acute bleeding complications in patients after hematopoietic stem cell transplantation with prophylactic platelet transfusion triggers of 10 x 10(9) and 20 x 10(9) per L. Transfusion. 2007 May;47(5):801-12.
116. Nevo S, Fuller AK, Zahurak ML, Hartley E, Borinsky ME, Vogelsang GB. Profound thrombocytopenia and survival of hematopoietic stem cell transplant patients without clinically significant bleeding, using prophylactic platelet transfusion triggers of 10 x 10(9) or 20 x 10(9) per L. Transfusion. 2007 Sep;47(9):1700-9.
117. Benson K, Fields K, Hiemenz J, Zorsky P, Ballester O, Perkins J, et al. The platelet-refractory bone marrow transplant patient: prophylaxis and treatment of bleeding. Seminars in oncology. 1993 Oct;20(5 Suppl 6):102-9.
118. Benson K, Marks AR, Marshall MJ, Goldstein JD. Fatal graft-versus-host disease associated with transfusions of HLA-matched, HLA-homozygous platelets from unrelated donors. Transfusion. 1994 May;34(5):432-7.
119. Brubaker DB. Clinical significance of white cell antibodies in febrile nonhemolytic transfusion reactions. Transfusion. 1990 Oct;30(8):733-7.
120. Decary F, Ferner P, Giavedoni L, Hartman A, Howie R, Kalovsky E, et al. An investigation of nonhemolytic transfusion reactions. Vox sanguinis. 1984;46(5):277-85.

121. Heddle NM, Klama LN, Griffith L, Roberts R, Shukla G, Kelton JG. A prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusions. Transfusion. 1993 Oct;33(10):794-7.
122. Kleinman S, Chan P, Robillard P. Risks associated with transfusion of cellular blood components in Canada. Transfusion medicine reviews. 2003 Apr;17(2):120-62.
123. Kuehnert MJ, Roth VR, Haley NR, Gregory KR, Elder KV, Schreiber GB, et al. Transfusion-transmitted bacterial infection in the United States, 1998 through 2000. Transfusion. 2001 Dec;41(12):1493-9.
124. Larsson LG, Welsh VJ, Ladd DJ. Acute intravascular hemolysis secondaryto out-of-group platelet transfusion. Transfusion. 2000 Aug;40(8):902-6.
125. Lucas G, Rogers S, Evans R, Hambley H, Win N. Transfusion-related acute lung injury associated with interdonor incompatibility for the neutrophil-specific antigen HNA-1a. Vox sanguinis. 2000;79(2):112-5.
126. McCullough J. Current issues with platelet transfusion in patients with cancer. Seminars in hematology. 2000 Apr;37(2 Suppl 4):3-10.
127. Muylle L, Wouters E, Peetermans ME. Febrile reactions to platelet transfusion: the effect of increased interleukin 6 levels in concentrates prepared by the platelet-rich plasma method. Transfusion. 1996 Oct;36(10):886-90.
128. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. The New England journal of medicine. 1996 Jun 27;334(26):1685-90.
129. Virchis AE, Patel RK, Contreras M, Navarrete C, Kaczmarski RS, Jan-Mohamed R. Lesson of the week. Acute non-cardiogenic lung oedema after platelet transfusion. BMJ (Clinical research ed. 1997 Mar 22;314(7084):880-2.