Platelet transfusions and patient outcomes after cardiac surgery

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Safety and efficacy of platelets concentrates and the impact of their storage conditions in cardiac surgery patients

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"Enkel met het hart kan men goed zien,
het essentiële is onzichtbaar voor de ogen"
Antoine de Saint-Exupéry

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CHAPTER 1

1

General introduction and outline of this thesis

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Part I: Platelet transfusions in general

Platelet transfusions

Platelet transfusions are used to provide hemostatic capacity to patients with decreased number or functionality of platelets.[1] In the Netherlands pooled platelet concentrates are prepared using five AB0 identical and Rh-D compatible buffy coats from whole blood donations and the remaining 10% of platelet units are collected by apheresis.[2] The five buffy coats for the pooled platelet units, each containing 25 mL of plasma, are resuspended either in 100% plasma of one of the five donors (plasma-platelets) or in 60-70% platelet additive solution (PAS) and 30-40% plasma (PAS-platelets).

Storage medium

PAS is a saline based crystalloid developed for the storage of platelets.[3] During this execution of this thesis PAS and plasma were used concurrently as storage medium for platelets in the Netherlands. In which storage medium platelets were stored was determined by the geographic location of a hospital. In the region South-West platelets were stored in PAS and in the other regions platelets were stored in plasma. Up to 2012 PAS-B fluid (T-sol, Baxter) and since 2013 PAS-C (Intersol, Fenwal, Inc)was used. In PAS-C phosphate is added as an extra buffer compared to PAS-B.[4]

PAS was developed to replace part of the plasma from platelet concentrates because of the considered harmful effect on platelets of enzymes in plasma.[5] Furthermore, with PAS it is possible to optimize the storage conditions, especially by increasing the buffer capacity and with that improving platelet quality.[6] Platelets use oxidation of glucose for energy supply which results in ATP and lactic acid which causes lowering of the pH. Lowering of the pH induces (more) platelet activation and thereby more glucose oxidation and resulting lactic acid production, which forms a vicious circle. Most types of PAS contain acetate as energy source for platelets to reduce the oxidation of glucose into lactic acid. Metabolism of acetate results in the formation of bicarbonate, which forms an extra buffer to stabilize the pH. All PAS-platelet units still contain 20-35% of plasma as source of glucose and to maintain platelet membrane integrity.[3,4,7]. In addition, several studies have suggested that cytokines and other substances present in the plasma fraction of platelet units play an important role in the etiology of transfusion reactions.[8,9] It has been suggested that reducing the amount of plasma in platelet units could give fewer transfusion reactions.

Indeed a number of studies have suggested that the use of PAS as storage medium significantly decreases the transfusion reaction rate, especially that of allergic reactions,

after platelet transfusion.[10-14] However, the majority of these studies concern transfusion of apheresis platelets to hematologic patients, so the results of these previous studies may not be applicable to other patients, like trauma or cardiac surgery patients, receiving platelet transfusions. In cardiac surgery patients the cardiopulmonary bypass results in an inflammatory response, endothelial dysfunction and vasoplegia which might influence the occurrence of transfusions reactions.[15,16] Thus, it is unclear whether pooled, buffy-coat-derived platelet units in PAS lead to fewer transfusion reactions compared to platelet units in plasma in the entire population of patients transfused with platelets.

In addition to the possible difference between PAS-platelets and plasma-platelets with regard to safety aspects like transfusion reactions, also the efficacy is of major concern. It is unclear whether PAS-platelet concentrates are as effective as plasma-platelet concentrates. A large in vitro study, comparing platelets in plasma with platelets stored in different PASs for 8 days, demonstrated inferior results for PAS-platelets compared to plasma-platelets, with regard to pH, lactate production, Annexin A5 binding and CD62P expression.[17] However, testing in this study testing was performed in the absence of red blood cells and blood plasma. Furthermore, the correlation between the analyzed in-vitro outcomes and clinically relevant endpoints has not been established.

The clinical studies analyzing the effectiveness of PAS-platelet transfusions have been performed in hematologic patients and have shown conflicting results.[11,18] The results of these hematological studies are not applicable to surgical and trauma patients, in whom considerable volume replacement can occur. In these previous studies the corrected count increment (CCI) is used as a measure for the efficacy of platelet transfusions. While the absolute count increment expresses the absolute increase in platelet count after transfusion, the corrected count increment (CCI) takes the platelet dose and the body surface area of the patient into account. So the CCI only provides information on the platelet count (in a stable blood volume / non-bleeding patient) and does not provide any information on the platelet function of the transfused platelets. It is not clear whether and to what extent CCI is correlated to clinical endpoints such as bleeding and applicable as an adequate surrogate endpoint.[19]

In contrast, multiple electrode aggregometry (Multiplate) is a platelet function test reported to correlate well with clinically relevant outcomes like bleeding and thromboembolic events in different clinical settings.[20-23] The Multiplate is a point-of-care (POC), impedance aggregometer which measures platelet aggregation in whole-blood samples using several agonists. Another clinically frequently used assay that has a clear correlation with clinical endpoints is thromboelastography (TEG) which is a whole-blood POC test assessing overall clot formation.[24,25]

Storage time

Besides storage medium also the storage time of platelet concentrates is of interest with regard to safety and efficacy of platelet transfusions. Platelet products are stored for a maximum of 4–7 days, depending on national guidelines and type of product.[26-28] Storage time has been associated with the accumulation of biological response modifiers, such as inflammatory cytokines and chemokines.[29-32] Whether these changes also have clinical consequences is not clear yet, as published results are contradictory. A review paper concluded that the risk of transfusion reactions was similar in old compared to fresh leukoreduced units,[28] whereas a more recent study showed that prolonged storage of platelets was associated with a higher frequency of inflammatory reactions, but not of allergic reactions.[33]

In vitro studies show that during storage platelets undergo multiple changes in structure and function collectively known as the "platelet storage lesion".[34,35] In patients this "platelet storage lesion" may result in a reduced hemostatic capacity.[36-38]

Part II: Platelet transfusions in cardiac surgery patients

Impact platelet transfusion in cardiac surgery

A significant part of all platelet transfusions is received by cardiac surgery patients.[39] Cardiac surgery carries a high risk for blood loss and blood transfusion due to invasiveness of the procedures and platelet dysfunction secondary to exposure to cardiopulmonary bypass (CPB), hemodilution and the use of (more and more) potent anti-platelet drugs.[40-43] Significant variation, ranging between 1.4% and 24.7% for isolated CABG, exists in platelet transfusion rates between countries, institutions and physicians.[44-46] This variety indicates inappropriate under- and/or overutilization of platelet transfusion and illustrates the lack of consensus on the indication for a platelet transfusion in certain clinical situations. In part this is explained by the platelet transfusions that are administered outside the (national) guidelines and without documented indication, as shown in an national audit in the UK.[47] Furthermore, the existing guidelines are not specific enough and do not cover several aspects of the clinical situation(s).[47] The American Association of Blood Banks (AABB) guidelines provide limited guidance regarding platelet transfusions in cardiac surgery. The following indications for platelet transfusion are mentioned: life-threatening active bleeding, platelet count less than 50,000/microliter in patients undergoing major surgery, and platelet count less than 10,000/microliter.[48] They caution against prophylactic preoperative transfusion of platelets in most instances, but note that transfusion may be appropriate in instances where there is perioperative bleeding and clinical suspicion for platelet dysfunction. In the 2017

EACTS/EACTA guidelines on platelet transfusions state that platelets should be transfused in bleeding patients with a platelet count below 50,000/microliter or patients on antiplatelet therapy with bleeding complications. (class of recommendation IIa, level of evidence C)[49] However, these guidelines do not provide clarity about several aspects of perioperative bleeding nor do they specify how to determine or confirm platelet dysfunction. This is because evidence regarding these clinical topics is lacking. Unlike for red blood cell transfusions, there are no studies available in which a certain platelet count or platelet function value served as a threshold for platelet transfusion and could be identified as the key effector to stop/reduce perioperative bleeding.[50] Moreover, there is a lack of clinical evidence establishing the hemostatic effect(iveness) of platelet transfusions in cardiac surgery patients.[51]

In addition, conflicting results have been reported regarding the safety of platelet transfusions in the setting of cardiac surgery as some studies have suggested that platelet transfusion may be associated with increased morbidity and mortality, while others have shown no difference with and without platelet transfusion with regard to safety.[52-58] Thus there is an unmet need to determine the clinical impact (safety and efficacy) of perioperative platelet transfusions in patients undergoing cardiac surgery.

Impact storage time platelets in cardiac surgery patients

As mentioned before *in vitro* studies show that during storage platelets undergo multiple changes in structure and function collectively known as the "platelet storage lesion".[34,35] It is conceivable that in patients this "platelet storage lesion" results in a reduced hemostatic capacity and more adverse events.[36-38] A recent review showed that transfusion of older platelets was associated with a shorter time to the next transfusion, a trend towards a higher risk of bleeding, and in hematology patients an increased need of platelet transfusions.[28] However, most clinical studies have been performed in non-bleeding hematology patients. Their results may not be applicable to cardiac surgery patients who have different needs (to stop or educe bleeding), different circumstances (like the use of antiplatelet medication and cardiopulmonary bypass) and platelet transfusion may have a different effect.

Aim and outline of this thesis

The aim of this thesis was to expand knowledge about the safety and efficacy of platelet transfusions in general and in particular in cardiac surgery patients by studying the influence of one unit of platelets, the influence of storage medium and the influence of storage time on clinical outcomes of patients. Expansion of knowledge about this topic can create possibilities to further improve the safety and efficacy of platelet transfusions.

This thesis starts with the question whether certain aspects of a platelet unit influence its efficacy and safety. It has been suggested that reducing the amount of plasma in platelet units could give fewer transfusion reactions. However, the results of these studies may not be applicable to other patients receiving platelet transfusions, like trauma or cardiac surgery patients. In the **second chapter** of this thesis we compared patients transfused with PAS-B-platelets, PAS-C-platelets, or plasma-platelets with regard to the occurrence of transfusions reactions in all types of patients transfused with platelets in routine clinical use.

In addition to the possible difference between PAS-platelets and plasma-platelets with regard to safety aspects like transfusion reactions, also the efficacy is of major concern. It is unclear whether PAS-platelet concentrates are as effective as plasma-platelet concentrates. **Chapter three** shows an in-vitro study comparing the hemostatic function of PAS-platelets to plasma-platelets in reconstituted whole blood. The hemostatic function is measured using Multiplate-derived platelet aggregation and TEG-measured overall clot formation.

Besides storage medium also the storage time of platelet concentrates is of interest with regard to safety and efficacy of platelet transfusions. Storage time has been associated with the accumulation of biological response modifiers, such as inflammatory cytokines and chemokines.[29-32]. Whether these changes also have clinical consequences is not clear yet, as published results are contradictory.[28,33] This controversy indicates that a better understanding of the influence of storage time on safety is needed, and will create an opportunity to further improve platelet transfusions. Therefore the study presented in the **fourth chapter** of this thesis assessed whether there is an association between storage time of (leuko-reduced pooled buffy-coat) platelets and transfusion reactions.

In addition to the characteristics of the platelet concentrate it is plausible that also the patient characteristics and the clinical situation influence the effect of a platelet transfusion. Since a significant part of platelet transfusions is consumed by cardiac surgery patients, it is important to understand the effect of a platelet concentrate and its storage conditions on cardiac surgery patients. There is a lack of clinical evidence establishing the hemostatic effect(iveness) of platelet transfusions in cardiac surgery

patients.[51] In addition, conflicting results have been reported regarding the safety of platelet transfusions in the setting of cardiac surgery.[52-58] So there is an unmet need to determine the clinical impact (safety and efficacy) of perioperative platelet transfusions in patients undergoing cardiac surgery. In the **fifth chapter** we study the efficacy and safety of platelet transfusion by comparing patients who received a platelet transfusion during cardiac surgery with propensity-score-matched patients who did not receive a transfusion.

As mentioned before *in vitro* studies show that during storage platelets undergo multiple changes in structure and function collectively known as the "platelet storage lesion".[34,35] It is concerned that in patients this "platelet storage lesion" results in a reduced hemostatic capacity and more adverse events.[36-38] Subsequently, the question arose whether this "platelet storage lesions" have clinical significant impact. In the **sixth chapter** we present our study analyzing whether platelet storage time is associated with efficacy and safety outcomes in cardiac surgery patients.

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PART I

Platelet transfusions in general

CHAPTER 2

2

Transfusion reactions after transfusion of platelets stored in PAS-B, PAS-C or plasma: a nationwide comparison

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Background

Platelets (PLTs) stored in PLT additive solution (PAS) are associated with fewer allergic reactions than plasma-stored PLTs. However, earlier studies could not provide conclusive evidence on febrile reactions, and did not analyze other transfusion reactions separately due to limited sample size. We therefore compared incidences of all transfusion reactions of PAS-B- PLTs, PAS-C- PLTs and plasma- PLTs.

Study design and methods

In this observational study, all transfusion reactions reported to the national hemovigilance office of the Netherlands from 2006 to 2015 were included.

Results

During the study period, a total of 2,407 transfusion reactions after PLT transfusions were reported. In that period 553,267 pooled buffy coat-derived PLT units were issued, of which 83,884 were stored in PAS-B, 45,728 in PAS-C and 423,655 in plasma. Regarding transfusion-related circulatory overload, transfusion-related acute lung injury, and "other reactions" no statistically significant differences were observed between the PLT products. When PAS-B-PLT transfusions were compared to plasma-PLT transfusions, the overall relative risk (RR) of transfusion reactions was 0.99 (95% confidence interval: 0.88;1.11); for allergic and febrile non-hemolytic transfusion reactions (FNHTRs) it was 0.66 (0.55;0.80) and 1.54 (1.27;1.86), respectively. When PAS-C-PLTs were compared to plasma-PLTs, the RR was 0.56 (0.46;0.68) for all transfusion reactions; 0.38 (0.28;0.52) for allergic reactions and 0.82 (0.59;1.13) for FNHTRs. When PAS-C-PLTs were compared to PAS-B-PLTs, for all reactions the RR was 0.56 (0.45;0.70); for allergic reactions 0.58 (0.40;0.82) and for FNHTRs 0.53 (0.37;0.75).

Conclusions

PAS-C-PLTs are associated with fewer transfusion reactions compared to plasma-PLTs and compared to PAS-B-PLTs.

Abstract

Introduction

Platelet (PLT) transfusions are used to provide hemostatic capacity to patients with a decreased number or functionality of PLTs.[1] Besides an improved hemostatic capacity, PLT transfusions can also cause transfusion reactions.[2] All transfusion reactions cause some degree of inconvenience for patients, involve increased costs, and potentially result in (severe) morbidity or death. Therefore, efforts, such as leukoreduction and the use of PLT additive solution (PAS), have been made to reduce the occurrence of transfusion reactions over the past decades.[3]

Previous studies have suggested that cytokines and other substances present in the plasma fraction of PLT units play an important role in the etiology of transfusion reactions.[4,5] It has been suggested that reducing the amount of plasma in PLT units could give fewer transfusion reactions. Washing and concentrating the PLTs are performed to decrease the volume of plasma in PLT units, but these are relatively time-consuming and may adversely affect PLT quality.[6-9] PLT additive solution was developed to replace part of the plasma for storage of PLTs. Earlier studies have suggested that the use of PAS as storage medium significantly decreases the transfusion reaction rate, especially that of allergic reactions, following PLT transfusion.[9-13] However, as the majority of studies analyzed data on apheresis PLT units and were performed in hematologic patients, the results of these studies may not be applicable to all patients receiving PLT transfusions. One study included all types of patients, but in this study only apheresis PLTs in PAS-C were transfused.[10] Thus, it is unclear whether pooled, buffy coat-derived PLT units in PAS-B or PAS-C lead to fewer transfusion reactions in a general patient population compared with PLT units in plasma.

In the Netherlands, PLT concentrates stored in both plasma and PAS are used, based on the geographical location of the hospital. This allowed us to validly compare the PAS-B-PLTs, PAS-C-PLTs and plasma-PLTs in one country with one central blood bank and one hemovigilance organization. The objective of this study was to compare PAS-B-PLTs, PAS-C-PLTs and plasma-PLTs with regard to the occurrence of anaphylactic and other allergic reactions, febrile non-hemolytic reactions (FNHTRs), transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI) in routine clinical use.

Material and methods

This nationwide, observational cohort study evaluated the impact of PLT storage medium (PAS-B, PAS-C and plasma) on transfusion reaction rates in the 10-year period from

2006 to 2015. Anonymized data were obtained from the national hemovigilance organization 'Transfusion and Transplantation Reactions in Patients' (TRIP) and the national Sanquin database (eProgesa, MAKsystems, Paris, France).

PLT products

All PLT products, pre-storage leuko- and plasma-reduced, are produced and stored by the Dutch blood bank Sanquin according to national and international standards. Approximately 90% of PLT concentrates are prepared using five ABO identical and Rh-D compatible buffy coats from whole blood donations. These five buffy coats, each containing 25 mL of plasma, are resuspended either in plasma of one of the five donors (plasma-PLTs) or in PLT additive solution (PAS-PLTs). Two types of PAS were used during the studied period: PAS-B (also known as PAS-2 or T-Sol, Baxter (Nivelles, France), Jan 1, 2006 - Nov 30, 2012) and PAS-C (also known as PAS-III or Intersol, Fenwal, a Fresenius company, La Châtre, France) Dec 1, 2012 - Dec 31, 2015). The remaining 10% of PLT units are collected by apheresis. Apheresis units, as well as hyperconcentrated PLT units were excluded from this analysis, since these are transfused for specific indications, including transfusion reactions that were the study objective of this analysis, which would have introduced bias. The total number of issued units per type of PLT product was obtained from the national Sanquin database (eProgresa).

Hemovigilance system

TRIP is the hemovigilance system in the Netherlands that has been operational since 2003. Participation of a hospital is regarded as the professional standard both in the national transfusion guideline and by the Healthcare Inspectorate. [14] Since 2008, in accordance with European legislation, the reporting of serious reactions to TRIP in parallel to the Healthcare Inspectorate as competent authority has been mandatory. Participation by the hospitals has been over 95% each year from 2006.[15] The definitions of reportable reaction types, severity and imputability are described in the annual TRIP report and website. These are similar to the international definitions developed by the International Haemovigilance Network and the hemovigilance working party of the International Society of Blood Transfusion. [16] In these definitions, FNHTRs and mild FNHTRs are collected separately, with FNHTRs being characterized by a temperature rise of ≥ 2°C and/or rigors, and mild FNHTRs by a temperature rise ≥1 and <2°C without rigors. For the comparison of the PLT products mild FNHTRs were not included, as not all hospitals report the mild FNHTRs. However, the numbers of mild FNHTRs are shown in the tables. The reaction type "other reaction" is a collection of reactions that do not fit in the definition of one of the reaction categories defined by TRIP.

Transfusion reactions are classified according to severity in five categories: [16,17]

- Grade 0: no morbidity
- Grade 1: minor morbidity, not life-threatening
- Grade 2: moderate to serious morbidity, may or may not be life-threatening; or leading to hospitalization or prolongation of illness; or associated with chronic disability or incapacity
- Grade 3: serious morbidity, directly life-threatening
- Grade 4: mortality following a transfusion reaction

The probability that a transfusion was responsible for the transfusion reaction is scored in the following imputability grades: "certain", "probable", "possible", "unlikely" and "excluded". [17]

Statistical analyses

General

The statistical analysis plan was reviewed by the departmental review committee before performing the actual analysis. All transfusion reactions in which a pooled buffy coat-derived PLT unit was involved, and which had been reported to TRIP between January 1, 2006, and December 31, 2015, were evaluated. Reactions with an imputability "unlikely" were included in the overall incidences, but not in the comparisons between the PLT products. In reaction types with fewer than five cases per group the overall incidences were reported, but no comparisons were made between the different PLT products. In addition, the reaction types anaphylactic and other allergic reaction were also evaluated as one category: allergic reactions. (Suspected) bacterial and viral transfusion-transmitted infections were included in the overall numbers, but were not evaluated separately as this information is reported elsewhere.[18]

Comparison of storage media

Only reactions with imputability "certain", "probable" or "possible" were included in the comparisons of transfusion reactions between the products. The transfusion reaction incidences of the three PLT storage media (PAS-B, PAS-C and plasma) were compared using logistic regression-derived odds ratios (ORs) with 95% confidence intervals (CIs). Due to the design of the study these odds ratios can be interpreted as risk ratios (RR).[19] Additionally, a sensitivity analysis was performed to assess whether transfusion of red blood cells (RBCs) or plasma disturb the results of the main analysis. In the sensitivity analysis transfusion reactions were excluded in which a combination of different types of blood products was involved.

Adjustment for potential confounding by centers

In the Netherlands, the type of storage medium of PLT units a hospital is supplied with is determined by the region. In one region hospitals are supplied with PAS-PLTs and in the other regions the hospitals are supplied with plasma-PLTs. In table S2 (available as supporting information in the online version of this paper) an overview can be found of all Dutch hospitals and the PLT product they are supplied with. This distribution of one or other type of PLT products to each of the hospitals may confound our results because the hospital determines both the type of PLT product and the reported transfusion reaction rate. The rate of reported transfusion reactions of hospitals may be influenced by the transfused blood product, but also by the transfusion policy and practice, and the patient population as well as the hospital's reporting instructions and discipline.

By weighting for hospital reporting rate, we reduced the potential confounding impact of the hospitals. As all Dutch hospitals use the same type of RBC units, the incidence of reported transfusion reactions after RBC transfusions was used as a measure for reporting tendency of the hospital. The incidences of non-university hospitals were grouped according to the PLT product they used (plasma-PLTs, PAS-B-PLTs or PAS-C-PLTs) because the number of transfusions per hospital was small. For university hospitals the incidences were calculated separately for every hospital. If a hospital switched to another PLT product separate incidences were calculated for the different periods. All RBC incidences of the hospital (group) were divided by the national RBC incidence to correct for reporting tendency, and then the inverse of this ratio was used as a weight. The transfusion reactions were weighted by these calculated inverses in our analyses. The incidences of transfusion reactions were thus weighted according to the following equation:

$$\textit{weighted incidence per center} = \frac{\textit{unweighted incidence per center}}{\textit{(center RBCs transfusion reaction incidence / national RBCs transfusion reaction incidence)}}$$

Results

General

Between 2006 and 2015, a total of 553,267 pooled buffy coat PLT units were issued in the Netherlands of which 83,884 were stored in PAS-B, 45,728 in PAS-C and 423,655 in plasma. In that period, 2,407 (0.43%) reactions involving PLTs were reported. For each transfusion reaction type, Table 1 shows the number of reported cases and proportion of the total number of transfusion reactions. Additionally, overall incidences for each reaction type are presented. The most frequent reactions were allergic transfusion

Table 1 Incidence of reported transfusion reactions

Reaction type	Number	Incidence (per 10,000)	% of total
Allergic reaction	1,144	21	47.5
• Anaphylactic reaction	262	5	10.9
• Other allergic reaction	882	16	36.6
FNHTR overall	842	15	35.0
• FNHTR	684	12	28.5
• Mild FNHTR	158	3	6.6
TACO	58	1	2.4
TRALI	44	1	1.8
Acute hemolytic reaction	8	0.1	0.3
Delayed hemolytic reaction	4	0.1	0.2
PTP	3	0.1	0.1
(Suspected) infection*	70	1	2.9
Other reaction	234	4	9.7
Overall	2,407	43	100

Data reported are absolute numbers, incidences (per 10,000), calculated by dividing the number of reactions by the total number of PLT units, and the percentage of each reaction type among all reactions.

* (Suspected) transfusion-transmitted bacterial/viral infection

FNHTR non-hemolytic transfusion reaction; PTP post-transfusion purpura; TACO transfusion-associated circulatory overload; TRALI transfusion-related acute lung injury

reactions with an overall incidence of 0.21%, and non-hemolytic transfusion reactions with an overall incidence of 0.15%. A few cases of hemosiderosis and delayed hemolytic reaction were reported, but all these cases were preceded by transfusion of not only PLTs, but also plasma and/or RBC. The majority, 2,085 of 2,407 (86.8%) of the reported reactions were classified as severity grade 1. In total, 268 reactions were associated with serious morbidity (grade 2 or higher), of which 14 had a fatal clinical outcome (grade 4).

Transfusion reactions in PLTs in PAS-B, PAS-C and plasma

The absolute numbers, the incidences and the RRs of the crude unweighted analysis are shown in Table 2, and the RRs resulting from the weighted main analysis are shown in Table 3. When PAS-B-PLTs were compared to plasma-PLTs in the weighted analysis the RR was 0.56 (95%CI 0.37;0.84) for anaphylactic reactions; 0.69 (0.56;0.85) for other allergic reactions and 1.54 (95%CI 1.27;1.86) for FNHTR. When PAS-C-PLTs were compared to plasma-PLTs the RR was 0.27 (95%CI 0.18;0.41) for other allergic reactions, and no difference was demonstrated regarding FNHTRs and anaphylactic reactions. When PAS-C-PLTs were compared to PAS-B-PLTs the RR was 0.53 (95%CI 0.37;0.75) for FNHTRs and 0.39 (95%CI 0.25;0.62) for anaphylactic reactions, but for other allergic reactions no

Table 2 Crude comparison of incidence of transfusion reactions across different storage media

	Number and incidence †						Risk ratio (95%CI) ‡		
PLTs in	PAS-B n=83,884		PAS-C n=45,728		plasma n=423,655		PAS-B vs plasma	PAS-C vs plasma	PAS-C vs PAS-B
Allergic reaction	116	(0.14)	50	(0.11)	978	(0.23)	0.60 (0.49;0.73)	0.47 (0.36;0.63)	0.79 (0.57;1.10)
• Anaphylactic reaction	23	(0.03)	14	(0.03)	225	(0.05)	0.52 (0.34;0.79)	0.58 (0.34;0.99)	1.12 (0.58;2.17)
• Other allergic reaction	93	(0.11)	36	(0.08)	753	(0.18)	0.62 (0.50;0.77)	0.44 (0.32;0.62)	0.71 (0.48;1.04)
FNHTR overall	169	(0.20)	64	(0.14)	609	(0.14)	1.40 (1.18;1.66)	0.97 (0.75;1.26)	0.69 (0.52;0.93)
• FNHTR	130	(0.15)	57	(0.12)	497	(0.12)	1.32 (1.09;1.60)	1.06 (0.81;1.40)	0.80 (0.59;1.10)
• Mild febrile reaction	39	(0.05)	7	(0.02)	112	(0.03)	NR	NR	NR
TACO	11	(0.01)	6	(0.01)	41	(0.01)	1.36 (0.70;2.64)	1.36 (0.58;3.19)	1.00 (0.37;2.71)
TRALI	10	(0.01)	0	(0)	34	(0.008)	1.49 (0.73;3.01)	NR	NR
Acute hemolytic reaction	1	(0.001)	0	(0)	7	(0.002)	NR	NR	NR
Delayed hemolytic reaction	2	(0.002)	0	(0)	2	(0.0005)	NR	NR	NR
PTP	0	(0)	1	(0.002)	2	(0.0005)	NR	NR	NR
(Suspected) infection*	10	(0.01)	4	(0.009)	55	(0.013)	NR	NR	NR
Other reaction	24	(0.03)	19	(0.04)	191	(0.05)	0.64 (0.42;0.97)	0.92 (0.58;1.48)	1.45 (0.80;2.65)
Overall	344	(0.41)	144	(0.31)	1,919	(0.45)	0.90 (0.80;1.01)	0.70 (0.59;0.82)	0.77 (0.64;0.94)

† absolute unweighted numbers and incidences per 100; **‡** risk ratios (95% confidence interval) of the two PAS-PLT types, both compared to plasma-PLTs, and of the PAS-C-PLTs compared to the PAS-B PLTs.

* (Suspected) transfusion-transmitted bacterial/viral infection

CI confidence interval; FNHTR non-hemolytic transfusion reaction; NR not reported; PAS PLT additive solution; PLTs PLTs; PTP post-transfusion purpura; TACO transfusion-associated circulatory overload; TRALI transfusion-related acute lung injury

Table 3 Comparison of transfusion reactions associated with PLTs in PAS-B, PAS-C and plasma, weighted for hospital reporting rates

	Risk ratio (95%CI)		
PLTs in	PAS-B vs plasma	PAS-C vs plasma	PAS-C vs PAS-B
Allergic reaction	0.66 (0.55;0.80)	0.38 (0.28;0.52)	0.58 (0.40;0.82)
• Anaphylactic reaction	0.56 (0.37;0.84)	0.73 (0.44;1.19)	1.32 (0.72;2.43)
• Other allergic reaction	0.69 (0.56;0.85)	0.27 (0.18;0.41)	0.39 (0.25;0.62)
FNHTR	1.54 (1.27;1.86)	0.82 (0.59;1.13)	0.53 (0.37;0.75)
TACO	1.34 (0.69;2.59)	1.59 (0.72;3.52)	1.19 (0.47;3.06)
TRALI	1.08 (0.46;2.50)	NR	NR
Other reaction	0.75 (0.49;1.15)	0.81 (0.47;1.39)	1.07 (0.55;2.08)
Overall	0.99 (0.88;1.11)	0.56 (0.46;0.68)	0.56 (0.45;0.70)

Risk ratios with 95% confidence interval, weighted for reporting rate.
CI confidence interval; FNHTR non-hemolytic transfusion reaction; NR not reported; PAS PLT additive solution; PLTs PLTs; PTP post-transfusion purpura; TACO transfusion-associated circulatory overload; TRALI transfusion-related acute lung injury

difference was demonstrated. Regarding TACO, TRALI and "other reactions" no statistically significant differences were observed between the different PLT products.

Sensitivity analyses

The results of the sensitivity analyses studying reactions where only PLT transfusions were involved are shown in Table S1 (available as supporting information in the online version of this paper). The RRs observed in these analyses for the different reaction types were similar to those found in the main analysis.

Discussion

In this nationwide study, PAS-C-PLTs were associated with a lower overall transfusion reaction incidence than both plasma-PLTs and PAS-B-PLTs. Furthermore, PAS-B-PLTs were associated with a lower incidence of allergic reactions, but had a higher incidence of FNHTR than plasma-PLTs. Regarding TACO, TRALI and "other reactions" no statistically significant difference was observed between the different PLT products.

Our finding that both PAS-B and PAS-C as storage fluid may decrease the risk of allergic reactions compared to plasma is consistent with results of studies on both pooled and apheresis PLTs.[10,11,13] It has been hypothesized that the replacement of most of the plasma, and thereby the amount of allergens in the PLT unit, reduces the risk of allergic

reactions.[20-22] PLT concentrates in PAS still require at least 30% plasma for the PLTs to maintain their quality, likely due to glucose and the buffering capacity of bicarbonate.[23,24] Likely, with the development of glucose- and bicarbonate-containing PASs,[25] the volume of plasma can be reduced further, potentially resulting in fewer allergic reactions. The comparison between PAS-B and PAS-C has not been made before, and our data show that PAS-C-PLTs have a 40% to 50% lower incidence of allergic reactions than PAS-B-PLTs. Although the concentrations of plasma were similar in both PASs, the allergic reaction rates in the PASs differed. This suggests that not only plasma and PLTs, but also the PAS fluid affects the chance of allergic reactions and furthermore that also the type of PAS influences the allergic reaction incidence. Elucidation of the underlying mechanism could benefit further reduction of the allergic reaction rate after PLT transfusions.

The incidence of FNHTRs was increased after transfusion of PAS-B-PLTs compared to plasma-PLTs. This finding is partially in agreement with two previous studies.[13,20] Both earlier studies showed no significant difference between the FNHTR rates of the two PLT products, probably because the patient populations were considerably smaller. However, the study in France did show a trend towards more FNHTRs in the pooled PLTs in PAS.[20] It is assumed that, besides other factors, PLT-derived soluble CD40L can cause FNHTR.[26,27] The release of soluble CD40L is induced by activation of PLTs during preparation and storage.[28,29] Transfusion of plasma-stored PLTs seems to lead to fewer FNHTRs than PAS-B-stored PLTs, which suggests that plasma is more capable of inhibiting PLT activation than PAS-B. When PAS-C-PLTs were compared to plasma-PLTs in our study, no difference was detected, which is consistent with one study but not with the other.[10,11] Furthermore, PAS-C-PLTs were compared to PAS-B-PLTs and demonstrated a lower incidence of FNHTR with the former. No previous literature was found on the clinical comparison of these products. The main difference in the composition of PAS-B and PAS-C is that, in contrast to PAS-B, PAS-C contains phosphate. The advantages of phosphate are that it supplies inorganic phosphate for ATP synthesis and that it functions as a buffer.[30] Possibly these two effects of phosphate in PAS-C explain the difference in FNHTRs between the PAS products. We postulate that better storage characteristics of PLTs in PAS-C, with less soluble CD40L release, are the explanation for our observation, but further in vitro studies need to be conducted.

To the best of our knowledge, this is the first study to directly compare PLTs stored in PAS-B, PAS-C and plasma concerning the less frequent reactions TACO, TRALI and anaphylactic reactions. This study hereby contributes relevant clinical knowledge as these less common reactions comprise more than 15% of all reactions and can lead to severe morbidity and even mortality. For TACO and TRALI, no significant differences were detected between the various PLT products. In contrast, for anaphylactic reactions

PLTs stored in PAS-B demonstrated a significantly lower incidence than PLTs in plasma. However, no significant difference was observed between PAS-C-PLTs and plasma-PLTs, which is remarkable as we expected an effect in the same direction as the PAS-B-PLTs. This may be the result of a lack of statistical power and warrants further study in a larger cohort.

The TRIP data are based on passive surveillance of transfusion reactions. This is both a limitation, because not all reactions are detected and reported, and a strength, because the reactions that are reported to TRIP are probably the most relevant reactions. The overall incidence of reported reactions in our study is relatively high compared to other countries using passive surveillance. As the incidence of transfusion reactions associated with red blood cells is also relatively high in the Netherlands, it is plausible that it reflects the accuracy of the hemovigilance system.[31,32] Another explanation for the relatively high incidence is that some other hemovigilance systems only report serious reactions. The main limitation of this study is that the use of either PAS-PLTs or plasma-PLTs is determined per hospital, so the reporting tendency of hospitals may be a confounder in this study. In order to reduce this confounding, the reactions were weighted, but residual confounding cannot be ruled out. An important strength of our study is that it spans a period of 10 years and is nationwide, which means that it covers all patients who were transfused with pooled buffy coat-derived PLTs. Moreover, our analysis included over half a million PLT transfusions, which made it possible to analyze less common reaction types like anaphylactic reactions, TRALI and TACO separately. Finally, it was possible to compare PLTs in plasma to both PLTs in PAS-B and PAS-C, which provides insights that assist in further improvement of PLT additive solutions. The sensitivity analysis suggests that the results of the main analysis are not affected by blood products other than PLTs.

In conclusion, PAS-C-PLTs appear to induce fewer transfusion reactions compared to plasma-PLTs and PAS-B-PLTs. Furthermore, PAS-B-PLTs are associated with fewer allergic reactions, but with more FNHTR than plasma-PLTs. While PAS has been used as a strategy to mitigate TRALI, our data do not allow any conclusions with respect to TRALI.

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Table S1 Comparison of transfusion reactions of platelets in PAS-B, PAS-C and plasma, weighted for hospital reporting rates (sensitivity analysis*)

		risk ratio (95%CI)	
Platelets in	**PAS-B vs plasma**	**PAS-C vs plasma**	**PAS-C vs PAS-B**
Allergic reaction	0.66 (0.54;0.80)	0.39 (0.28;0.54)	0.59 (0.41;0.85)
• Anaphylactic reaction	0.55 (0.36;0.86)	0.81 (0.50;1.31)	1.46 (0.78;2.71)
• Other allergic reaction	0.69 (0.55;0.85)	0.27 (0.17;0.41)	0.39 (0.24;0.63)
FNHTR	1.55 (1.26;1.90)	0.85 (0.61;1.20)	0.55 (0.38;0.80)
TACO	1.17 (0.47;2.92)	0.29 (0.03;2.84)	0.25 (0.02;2.72)
TRALI	0.30 (0.04;2.12)	NR	NR
Other reaction	0.80 (0.51;1.27)	0.54 (0.26;1.10)	0.67 (0.30;1.52)
Overall	0.97 (0.86;1.10)	0.52 (0.42;0.64)	0.53 (0.42;0.67)

Risk ratios with 95% confidence interval, weighted for reporting rate.

FNHTR non-hemolytic transfusion reaction; NR not reported; PTP post-transfusion purpura; TACO transfusion-associated circulatory overload; TRALI transfusion-related acute lung injury

* In the sensitivity analysis transfusion reactions were excluded when a combination of different types of blood products was involved.

Table S2 The platelet storage medium of the hospitals in the Netherlands

Hospital	Period	Storage medium
Academic Medical Center of Amsterdam	January 2006 - December 2015	plasma
Academic Hospital Maastricht	January 2006 - December 2015	plasma
Admiraal De Ruyter Hospital	January 2006 - December 2015	PAS
Albert Schweitzer Hospital	January 2006 - December 2015	PAS
Amphia Hospital	January 2006 - December 2015	PAS
Amstelland Hospital	January 2006 - December 2015	plasma
Antoni of Leeuwenhoek	January 2006 - December 2015	plasma
Antonius Hospital	January 2006 - December 2015	plasma
Atrium Medical Center Parkstad	January 2006 - December 2015	plasma
Bernhoven Hospital	January 2006 - December 2015	plasma
Bethesda Hospital	January 2006 - December 2015	plasma
Bovenij Hospital	January 2006 - December 2015	plasma
Bronovo Hospital	January 2006 - December 2007	plasma
Bronovo Hospital	January 2008 - December 2015	PAS
Canisius-Wilhelmina Hospital	January 2006 - December 2015	plasma
Catharina Hospital	January 2006 - December 2015	plasma
Christian Hospital Nij Smellinghe	January 2006 - December 2015	plasma
Deventer Hospitals	January 2006 - December 2015	plasma
Diaconessenhuis Leiden	January 2006 - December 2010	plasma
Diaconessenhuis Leiden	January 2011 - December 2015	PAS
Diaconessenhuis Meppel	January 2006 - December 2015	plasma
Diakonessenhuis	January 2006 - December 2015	plasma
Elkerliek Hospital	January 2006 - December 2015	plasma
Erasmus Medical Center	January 2006 - December 2015	PAS
FlevoHospital	January 2006 - December 2015	plasma
Franciscus Hospital	January 2006 - December 2015	PAS
Gelderse Vallei Hospital	January 2006 - December 2015	plasma
Gelre Hospitals	January 2006 - December 2015	plasma
Gemini Hospital	January 2006 - December 2015	plasma
Groene Hart Hospital	January 2006 - December 2015	PAS
Haga Hospital	January 2006 - December 2015	PAS
Haven Hospital	January 2006 - December 2015	PAS
Hofpoort Hospital	January 2006 - December 2015	plasma
Hospital De Tjongerschans	January 2006 - December 2015	plasma
Hospital Group Twente	January 2006 - December 2015	plasma
IJsselland Hospital	January 2006 - December 2015	PAS
Ikazia Hospital	January 2006 - December 2015	PAS
Isala Hospital	January 2006 - December 2015	plasma

Table S2 continued

Hospital	Period	Storage medium
Jeroen Bosch Hospital	January 2006 - December 2015	plasma
Kennemer Gasthuis	January 2006 - February 2009	plasma
Koningin Beatrix Hospital	January 2006 - December 2015	plasma
Laurentius Hospital	January 2006 - December 2015	plasma
Leiden University Medical Center	January 2006 - July 2014	plasma
Leiden University Medical Center	July 2014 - December 2015	PAS
Lievensberg Hospital	January 2006 - December 2015	PAS
Maasstad Hospital	January 2006 - December 2015	PAS
Maasstad Hospital	January 2006 - December 2015	PAS
Martini Hospital	January 2006 - December 2015	plasma
Máxima Medical Center	January 2006 - December 2015	plasma
Meander Medical Center Amersfoort	January 2006 - December 2015	plasma
Medial	January 2006 - December 2015	plasma
Medical Center Alkmaar	January 2006 - December 2015	plasma
Medical Center Haaglanden	January 2006 - December 2015	PAS
Medical Center Leeuwarden	January 2006 - December 2015	plasma
Medical Spectrum Twente	January 2006 - December 2015	plasma
Ommelander Hospital Group	January 2006 - December 2015	plasma
Onze Lieve Vrouwe Gasthuis	January 2006 - December 2015	plasma
Orbis Medical Center	January 2006 - December 2015	plasma
Radboud University Medical Center	January 2006 - December 2015	plasma
Refaja Hospital	January 2006 - December 2015	plasma
Reinier de Graaf Groep	January 2006 - December 2015	PAS
Rijnland Hospital	January 2006 - September 2008	plasma
Rijnland Hospital	September 2008 - December 2015	PAS
Rijnstate Hospital	January 2006 - December 2015	plasma
Rivas Zorggroep	January 2006 - December 2015	PAS
Rivierenland Hospital	January 2006 - December 2015	plasma
Rode Kruis Hospital	January 2006 - December 2015	plasma
Saxenburgh Groep	January 2006 - December 2015	plasma
Scheper Hospital	January 2006 - December 2015	plasma
Slingeland Hospital	January 2006 - December 2015	plasma
Slotervaart Hospital	January 2006 - December 2015	plasma
Spaarne Hospital	January 2006 - December 2015	plasma
Spijkenisse Medical Center	January 2006 - December 2015	PAS
St. Anna Hospital	January 2006 - December 2015	plasma
St. Antonius Hospital	January 2006 - December 2015	plasma
St. Antonius Hospital	January 2006 - December 2015	plasma
St. Elisabeth Hospital	January 2006 - December 2015	plasma

Table S2 continued

Hospital	Period	Storage medium
St. Franciscus Gasthuis	January 2006 - December 2015	PAS
St. Jansdal Hospital	January 2006 - December 2015	plasma
St. Lucas Andreas Hospital	January 2006 - December 2015	plasma
St. Maartenskliniek	January 2006 - December 2015	plasma
Synergos St. Jans Gasthuis	January 2006 - December 2015	plasma
t Lange Land Hospital	January 2006 - February 2009	plasma
t Lange Land Hospital	February 2009 - December 2015	PAS
Tergooi Hospitals	January 2006 - December 2015	plasma
Tweesteden Hospital	January 2006 - December 2015	plasma
University Medical Center Groningen	January 2006 - December 2015	plasma
University Medical Center Utrecht	January 2006 - December 2015	plasma
Van Weel Bethesda Hospital	January 2006 - December 2015	PAS
VieCuri Medical Center Noord-Limburg	January 2006 - December 2015	plasma
Vlietland Hospital	January 2006 - December 2015	PAS
VU Medical Center	January 2006 - December 2015	plasma
Waterland Hospital	January 2006 - December 2015	plasma
Westfriesgasthuis	January 2006 - December 2015	plasma
Wilhelmina Hospital Assen	January 2006 - December 2015	plasma
Zaans Medical Center	January 2006 - December 2015	plasma
Zorgsaam Zeeuws-Vlaanderen	January 2006 - December 2015	PAS

CHAPTER 3

3

Comparison of hemostatic function of PAS-C-platelets versus plasma-platelets in reconstituted whole blood using impedance aggregometry and thromboelastography

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Background and objectives

There are concerns about the hemostatic function of platelets stored in platelet additive solution (PAS). Aim of this study was to compare the hemostatic function of PAS-C-platelets to plasma-platelets in reconstituted whole blood.

Materials and methods

In our experiment, whole blood was reconstituted with red blood cells, solvent-detergent (SD) plasma, and either PAS-C-platelets or plasma-platelets (n=7) in a physiological ratio. On storage days 2, 5, 8 and 13 the agonist-induced aggregation (multiple electrode aggregometry), clot formation (thromboelastography), and agonist-induced CD62P responsiveness (flow cytometry) were measured.

Results

Samples with PAS-C-platelets showed significantly lower aggregation than plasma-platelets when induced with adenosine diphosphate, -6 U (95% confidence interval: -8;-4) or thrombin receptor activating protein, -15 U (-19;-10). Also when activated with collagen and ristocetin the PAS-C-platelets showed less aggregation, although not statistically significant. All samples with PAS-C-platelets showed significantly lower agonist-induced CD62P responsiveness than samples with plasma-platelets. However, there was no difference regarding all TEG parameters.

Conclusion

Our findings demonstrate that the function, aggregation and CD62P responsiveness, of PAS-C-platelets in reconstituted whole blood is inferior to that of plasma-platelets, which may have implications in the setting of massive transfusions.

Abstract

Introduction

Platelet transfusions are used to provide hemostatic capacity to patients with a decreased number or functionality of platelets. In the Netherlands both platelet concentrates stored in plasma and platelets stored in a mixture of plasma and platelet additive solution C (PAS-C) are used. Among others, an advantage of PAS-C-platelets is that they cause fewer transfusion reactions.[1, 2]

However, it is unclear whether PAS-C-platelets are as effective as plasma-platelets. A large *in vitro* study, comparing platelets in plasma with platelets stored in different PASs until storage day 8, demonstrated inferior results for PAS-C-platelets compared to plasma-platelets, with regard to pH, lactate production, Annexin A5 binding and CD62P expression.[3] This study was applied in platelet concentrates, in the absence of red blood cells and plasma. Furthermore, the correlation between the analyzed outcomes and clinically relevant endpoints has not been established.

The clinical studies analyzing the effectiveness of PAS-C-platelet transfusions have been performed in hematology patients and have shown conflicting results.[2, 4] The results of these hematological studies are not applicable to surgical and trauma patients, in whom considerable volume replacement is common. Moreover, in these studies the corrected count increment (CCI) is often used as a measure for the efficacy of platelet transfusions. However, the CCI does not provide information on the platelet function. Furthermore, CCI is not applicable in all clinical settings in which platelets are transfused and it is unclear whether and to what extent CCI is correlated with clinical endpoints such as bleeding.[5]

In contrast, multiple electrode aggregometry (Multiplate analyzer) is a platelet function test reported to correlate well with clinically relevant outcomes like bleeding and thromboembolic events in different clinical settings.[6-9] The Multiplate is a point-of-care (POC), impedance aggregometer which measures platelet aggregation in whole blood samples using several agonists. This test is increasingly used in daily clinical practice to assess the hemostatic condition of patients and to guide transfusion and other interventions. Another frequently used assay that has a clear correlation with clinical endpoints is thromboelastography (TEG) which is a whole blood POC test assessing overall clot formation.[10, 11]

The objective of this exploratory study was to compare the hemostatic function of PAS-C-platelets to plasma-platelets in reconstituted whole blood. To compare the *ex vivo* functionality of the two platelet products in a condition that is as comparable as possible to an *in vivo* transfusion, whole blood was reconstituted to create an *ex vivo* model.

The hypothesis is that Multiplate-derived aggregation, TEG-measured clot formation, and flow cytometric CD62P responsiveness of PAS-C-platelets are inferior to those of plasma-platelets in reconstituted whole blood.

Materials and methods

Blood products

All blood products were produced and stored by the Dutch Blood Bank Sanquin according to national standards. The platelet concentrates were prepared from five pooled buffy coats from five whole blood donations, and were pre-storage leukoreduced using filtration. The platelets were resuspended either in 100% plasma from one of the five whole blood donations (plasma-platelets) or in 65% platelet additive solution C (also known as PAS-III or Intersol, Fresenius Kabi, Emmer-Compascuum, The Netherlands) and 35% plasma (PAS-C-platelets). To establish a more accurate estimation of the trend over storage time the platelet concentrates were stored and analyzed for 13 days. However, the current maximal storage time in clinical practice is 7 days, so only the results of storage up to 7 days apply to clinical practice. Both the whole period of 13 days and the period up to 8 days are analyzed separately. The platelets were stored at 20-24°C under constant agitation in a PVC-butyryl trihexyl citrate (BTHC) container (C5000, Fresenius Kabi, Bad Homburg, Germany). To obtain RBC units whole blood was centrifuged to remove the buffy-coat and plasma where after RBCs were resuspended in 110 ml saline-adenine-glucose-mannitol (SAGM), and then filtered to remove leucocytes to obtain a standard RBC product. For this experiment the RBC units were stored less than 3 days, at a temperature of 2-6°C, before being mixed with the other components. To use a standardized product, the plasma product that was used was a pooled solvent-detergent-treated plasma (Omniplasma, Octapharma, Vienna, Austria). The plasma was stored in aliquots below -30°C until use.

Whole blood reconstitution

To compare the *ex vivo* functionality of the two platelet products in a condition that is as comparable as possible to an *in vivo* transfusion, whole blood was reconstituted to create an *ex vivo* model. The reconstituted blood samples were composed of AB0-matched RBCs, plasma, and either PAS-C-platelets (n=7) or plasma-platelets (n=7) (Figure S1a). On day 2, 5, 8 and 13 a sample was taken from the stored platelet concentrates to make reconstituted whole blood. The intended ratio of the blood components in the reconstituted whole blood samples was physiological, in other words, a platelet count of 200 * 10^9/l and a hematocrit of 40%. Based on the platelet count of the platelet unit, the volume necessary to achieve a platelet count of 200 * 10^9/l in the whole blood sample was calculated. A sample was taken from a unit of RBCs and centrifuged at 1851

g for 10 minutes (Rotina 420R, Hettich, Geldermalsen, the Netherlands) to reduce the amount of SAGM. The concentrated RBCs and plasma were mixed with the sample of the platelet unit in the aforementioned ratio. Subsequently, the platelet aggregation capacity, agonist-induced CD62P responsiveness and clot formation were assessed in the reconstituted whole blood samples with multiple electrode aggregometry (Multiplate analyzer, Roche Diagnostics Ltd, Rotzkreuz, Switzerland), flow cytometry (FACSCanto, BD Biosciences, Breda, the Netherlands) and thromboelastography (TEG, Haemonetics, Braintree, MA, USA) respectively. To check the composition of the reconstituted whole blood, the platelet concentration and hematocrit were determined (Sysmex XT2000i, TOA, Tokyo, Japan).

Multiple electrode aggregometry

The Multiplate was used to assess platelet function. Platelet aggregation was assessed in single-use test cells that incorporate two independent sensor units, on which platelet aggregation occurs. According to the manufacturer's recommendations all samples were recalcified except for the ristocetin-activated samples. Three hundred microlitres of the reconstituted whole blood was mixed with 300 µl 0.9% NaCl with 3 mmol/l $CaCl_2$ (or 0.9% NaCl in ristocetin-activated samples) in the test cell. After 3 minutes of incubation, 20 µl of activator was added to the test cell. Four different activators were used in each sample on every storage day: adenosine diphosphate (ADP; final concentration 6.5 µmol/ml) that stimulates the P2Y-receptor; ristocetin (RISTO; 0.77 mg/ml) that gives binding of von Willebrand factor to the GPIb receptor; collagen (COL; 3.2 µg/ml) which binds to integrin α2β1 and the GPVI receptor, and thrombin receptor-activating peptide (TRAP; 32 µmol/ml) which stimulates the proteinase-activated receptor 1. All reagents were obtained from the manufacturer. After 6 minutes, the area under the curve (AUC) was determined. All measurements were taken at 37^0C.

Thromboelastography

The clot formation of the whole blood samples was measured using TEG. All samples were measured according to the manufacturer's instructions using disposable cups, pins and standard citrated kaolin reagent from the manufacturer. The samples were recalcified before performing the citrate-kaolin test. Details have been described earlier. [12]

Agonist-induced CD62P expression

To analyze platelet responsiveness the agonist-induced CD62P expression was measured using flow cytometry. The method has been reported previously and was applied with the exception that convulxin (CVX; highest concentration of 39 nmol/l), was used instead of collagen-related peptide.[13] The "platelet responsiveness" was calculated by subtracting the percentage of platelets expressing CD62P with lowest agonist level from

the percentages of platelets expressing CD62P at each concentration, like described earlier.[13] Then the area under the curve (AUC) of these percentages of "platelet responsiveness" was determined (Figure S2). Secondly, the CD62P expression with lowest agonist level, "baseline CD62P expression", was compared between the platelet types. Finally, the CD62P expression with highest level of agonist, "maximal CD62P expression", was compared between platelet types.

Additional experiments

PAS-platelets and PAS fluid compared to plasma-platelets and plasma

To visualize not only the influence of the PAS-C-platelets but also the maximal impact of the accompanying PAS-C-supernatant on hemostatic function, an additional experiment was performed. This experiment, shown in Figure S1B of the Supporting Information, was similar to the main experiment expect for the SD plasma that was replaced entirely by platelet concentrate supernatant, either PAS-C or plasma (n=7). As this experiment focused on the influence of platelets and the accompanying storage fluid on the hemostatic function the clot formation was measured using TEG.

Biochemical characteristics of platelet units

To verify our results, we measured previously studied parameters in the platelet concentrates, that is, before the platelets were mixed with plasma and RBCs. On days 2, 5, 8 and 13 after donation the pH, pO2, pCO2, glucose, lactate, unstimulated CD62P expression and Annexin A5 binding were measured as described previously.[3]

Statistical analysis

The data in the figures are presented as mean and 95% confidence interval (CI). The mean platelet concentration and hematocrit of the whole blood samples prepared with PAS-C-platelets were compared with those of the samples containing plasma-platelets using an independent sample t-test. The aggregation and clot formation in the reconstituted whole blood samples over the whole storage time (13 days), were analyzed with generalized linear models with platelet type (PAS-C-platelets or plasma-platelets) and storage time (in days) as independent variables. The influence of the platelet type independent of storage time, and the influence of storage time independent of platelet type, were expressed as the regression coefficient (beta) with 95% CI (results shown in Table 1). If there was a significant difference between the platelet types, the potential interaction between platelet type and storage time was analyzed in a separate model, by adding an interaction term to the generalized linear model. As in clinical practice platelets are stored up to 7 days, additionally a sensitivity analysis was performed on the data of days 2,5 and 8 to check whether the results regarding the influence of the platelet type are in agreement with the analysis including day 13. The same generalized linear models as

Table 1 Influence of platelet type and storage time on platelet aggregation and clot formation

		PAS-C-platelets (vs. plasma-platelets)	storage time (per day)
Multiplate	ADP (U)	-5.6 (-7.7;-3.5)	-1.3 (-1.5;-1.0)
	COL (U)	-1.5 (-4.0;0.9)	-1.1 (-1.4;-0.8)
	RISTO (U)	-2.5 (-6.6;1.6)	-1.2 (-1.7;-0.7)
	TRAP (U)	-14.5 (-18.8;-10.1)	-2.2 (-2.7;-1.7)
TEG	MA (mm)	-0.6 (-3.5;2.2)	-0.1 (-0.4;0.3)
	R-time (min)	0.06 (-0.27;0.39)	-0.07 (-0.11;-0.03)
	angle (^{0})	0.1 (-1.6;1.9)	0.1 (-0.1;0.3)

Main analysis includes data up to storage day 13. Values are generalized linear model regression derived coefficients (95% confidence interval) of platelet type (mean difference between 2 platelet types) and storage time (mean change per day) on aggregation induced with ADP, COL, RISTO and TRAP, and clot strength (MA), initial clot formation (R-time) and clot growth rate (angle). n = 14; * n = 13.

described above were performed on the data of storage days 2, 5 and 8 (results shown in Table 2). Comparisons between the platelet types regarding the AUCs of the CD62P "platelet responsiveness", "baseline CD62P expression" and "maximal CD62P expression" were made with an independent sample t-test (results shown in Table 3).

Table 2 Influence of platelet type and storage time on platelet aggregation and clot formation

		PAS-C-platelets (vs. plasma-platelets)	storage time (per day)
Multiplate	ADP (U)	-5.9 (-8.4;-3.4)	-1.8 (-2.4;-1.3)
	COL (U)	-1.8 (-4.6;0.9)	-1.9 (-2.5;-1.3)
	RISTO (U)	-3.4 (-8.7;1.8)	-2.0 (-3.1;-0.9)
	TRAP (U)	-12.3 (-17.3;-7.3)	-2.5 (-3.5;-1.4)
TEG	MA (mm)	-1.3 (-4.8;2.2)	0.05 (-0.66;0.76)
	R-time (min)	0.04 (-0.39;0.47)	-0.10 (-0.18;-0.01)
	angle (^{0})	-1.1 (-3.0;0.8)	0.5 (0.1;0.8)

Sensitivity analysis includes data up to storage day 8. Values are generalized linear model regression derived coefficients (95% confidence interval) of platelet type (mean difference between 2 platelet types) and storage time (mean change per day) on aggregation induced with ADP, COL, RISTO and TRAP, and clot strength (MA), initial clot formation (R-time) and clot growth rate (angle). n = 14; * n = 13.

Table 3 CD62P expression (%) of plasma-platelets compared to PAS-C-platelets

agonist	day	responsiveness	baseline CD62P expression	maximal CD62P expression
ADP	2	125 (87;163)	-27 (-37;-16)	10 (0;20)
	5	81 (61;101)	-26 (-33;-20)	4 (-5;12)
	8	90 (71;109)	-24 (-30;-18)	7 (1;13)
	13	55 (45;65)*	-6 (-14;2)*	12 (6;19)*
CVX	2	88 (50;127)	-26 (-37;-15)	0 (-1;1)
	5	109 (82;135)	-27 (-34;-20)	2 (1;3)
	8	103 (78;127)	-23 (-29;-17)	4 (3;6)
	13	64 (40;108)*	-5 (-13;3)*	13 (10;17)*
TRAP	2	125 (83;168)	-27 (-37;-16)	0 (-1;1)
	5	124 (91;158)	-27 (-34;-19)	2 (1;3)
	8	115 (93;137)	-23 (-30;-16)*	3 (2;5)
	13	79 (44;114)*	-5 (-13;3)*	12 (8;15)*

Values are mean difference (95% confidence interval) between plasma-platelets and PAS-C-platelets in reconstituted whole blood samples for CD62P responsiveness (%), "baseline CD62P expression": after stimulation with the lowest agonist level and "maximal CD62P expression": after stimulation with highest agonist level. N = 7 in both groups; * n = 6.

Results

Platelet aggregation (Multiplate)

The reconstituted whole blood with PAS-C-platelets had a mean platelet concentration of 183 (95% CI: 177;190) * 10^9/l and hematocrit of 37.9% (37.1;38.7%), which were not different from those in the plasma-platelets-samples: 182 (174;190) * 10^9/l platelets and a hematocrit of 37.3% (36.8;37.7%). The samples with PAS-C-platelets activated with ADP and TRAP showed significantly lower aggregation than the samples with plasma-platelets (Table 1). Although not statistically significant, when induced with COL and RISTO the PAS-C-platelets also showed less aggregation. In all samples with all activators the aggregation significantly declined over storage time (raw data are shown in Figure 1). The difference between the platelet types remained stable over storage time when aggregation was induced with ADP (interaction term -0.1 U,-0.6;0.5). However, TRAP-induced aggregation declined significantly faster in PAS-C-platelets than in plasma-platelets (interaction term: -1.2 U, -2.2;-0.1). The effect of platelet type detected in the sensitivity analysis was similar to that found in the analysis of 13 days of storage (results shown in Table 2).

Clot formation (TEG)

The clot strength (maximum amplitude, MA), initial clot formation (R-time) and clot growth rate (angle) of the samples with PAS-C-platelets were similar in the samples with

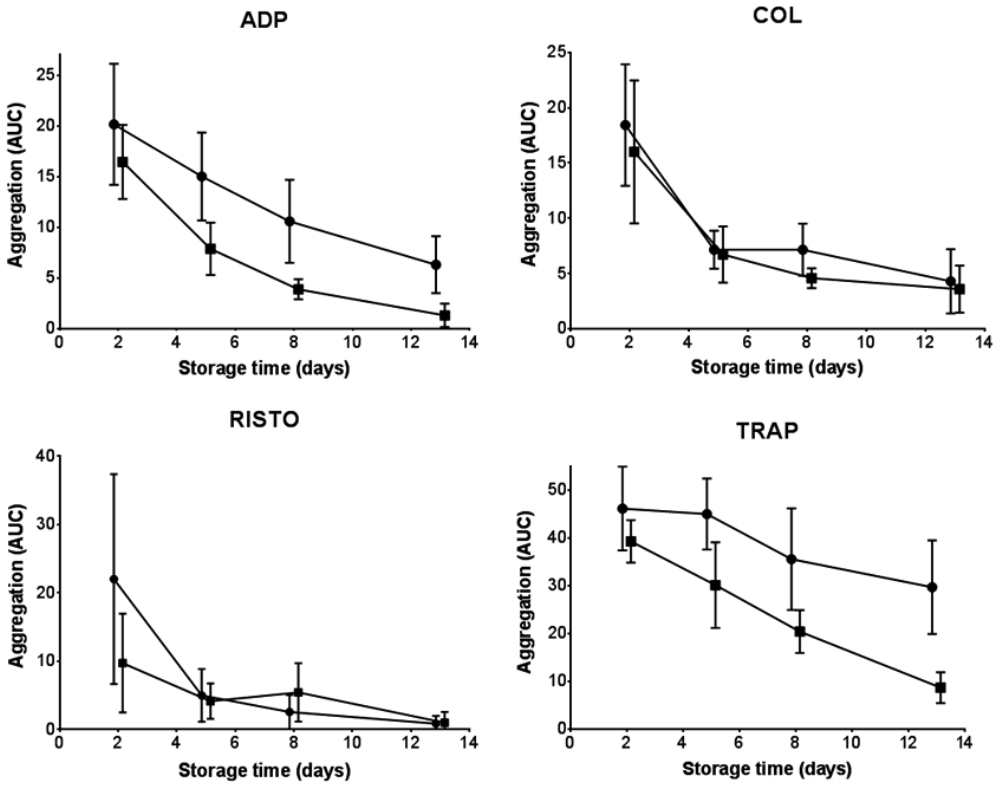

Figure 1 Aggregation (Multiplate) The mean aggregation of plasma-platelets (●) or PAS-C-platelets (■) in reconstituted whole blood, induced by ADP, COL, RISTO and TRAP. Error bars represent 95% confidence intervals.

plasma-platelets (Table 1). Initial clot formation significantly declined with increasing storage time, but maximum clot strength and clot growth rate remained rather constant with increasing storage time (raw data are shown in Figure S3A of the Supporting Information). The influence of platelet type on the clot formation found in the sensitivity analysis was similar to that found in the analysis of all 13 days of storage (results shown in Table 2).

CD62P responsiveness (flow cytometer)

The platelet CD62P responsiveness after stimulation with increasing concentrations of the agonists ADP, CVX and TRAP for samples containing PAS-C-platelets or plasma-platelets during 13 days of storage are shown in Figure 2. The CD62P expression with minimal agonist concentration, indicating baseline activation, was higher in samples with PAS-C-platelets than with plasma-platelets on every storage day for all three agonists (Table 3). With minimal agonist stimulation, both sample types showed a pattern

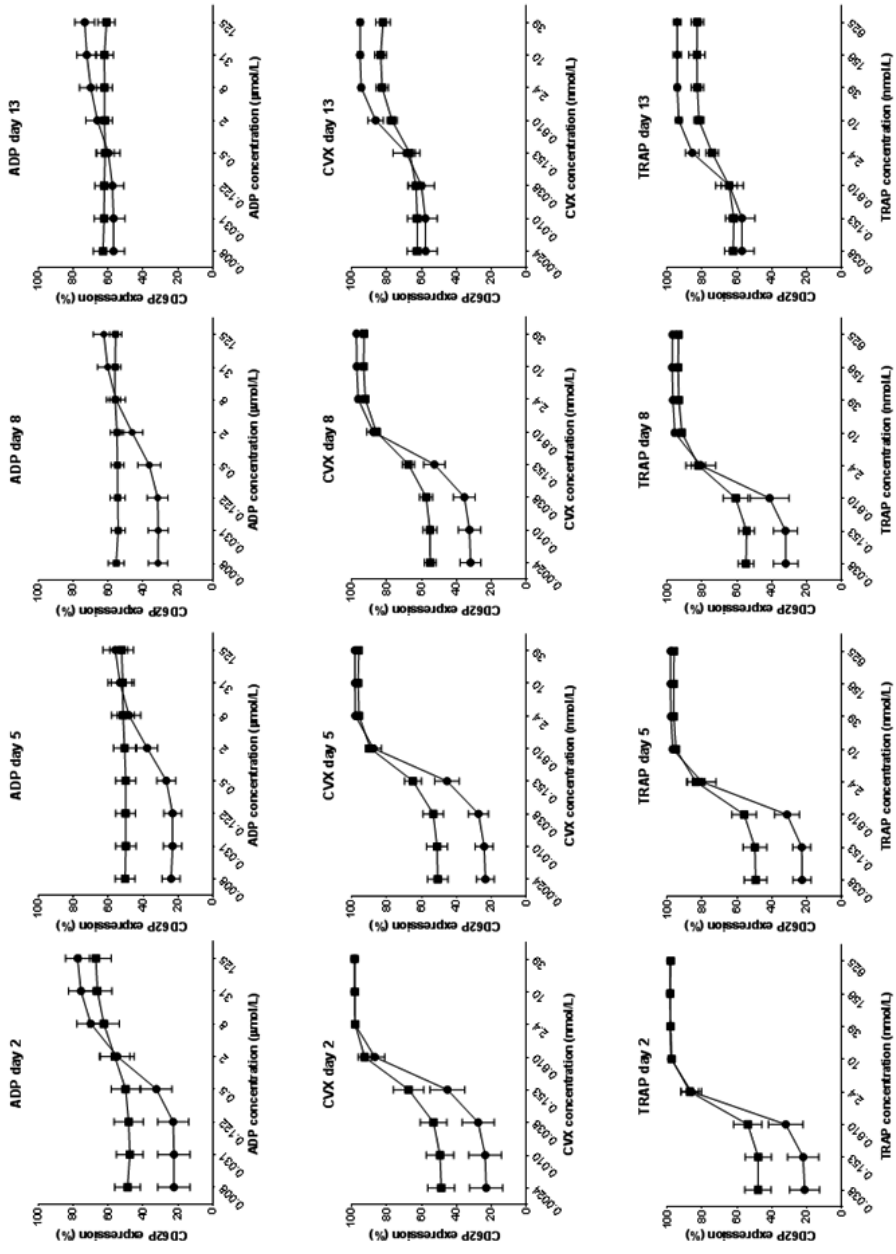

Figure 2 CD62P expression CD62P expression of plasma-platelets (●) or PAS-C-platelets (■) in reconstituted whole blood, induced by 8 different concentrations of ADP, convulxin (CVX) and TRAP on storage day 2, 5, 8 and 13

of increasing percentage of CD62P expression over storage time (raw data are shown in Figure 2). The CD62P expression with highest agonist concentration was lower in samples with PAS-C-platelets than with plasma-platelets on every storage day and with all three agonists (Table 3), suggesting a lower ability to respond to the platelet agonists. The calculated AUC, used as read-out for platelet responsiveness, was higher in samples with plasma-platelets than PAS-C-platelets, for all agonists (ADP, CVX and TRAP) and on all storage days (Table 3).

Additional experiments

PAS-platelets and PAS fluid compared to plasma-platelets and plasma in reconstituted whole blood

There was no (statistically) relevant difference between the mean platelet count and hematocrit of the samples containing PAS-C-platelets and accompanying PAS-C-supernatant and those with plasma-platelets and accompanying plasma. The samples containing both PAS-C-platelets and PAS-C-supernatant from the same unit showed a maximum amplitude that was on average lower, an R-time that was longer and an angle that was smaller than the samples with plasma-platelets and accompanying plasma when analyzed in the TEG (Figure S3B of the Supporting Information).

Biochemical characteristics of platelet units

The pH, pO_2, pCO_2, glucose, lactate, unstimulated CD62P expression and Annexin A5 binding of the platelet concentrates showed patterns comparable to earlier observed results (shown in Figure S4).

Discussion

The aggregation of PAS-C-platelets was lower than the aggregation of plasma-platelets. Also, the agonist-induced CD62P responsiveness of PAS-C-platelets was significantly lower than the responsiveness of plasma-platelets. Clot formation, as assessed with TEG, of the reconstituted whole blood showed no differences between the two platelet types.

To the best of our knowledge, this study is the first comparing the hemostatic function of PAS-C-platelets to plasma-platelets using whole blood platelet aggregometry and thromboelastography in reconstituted whole blood. By studying reconstituted whole blood instead of unmixed platelet concentrates, the interaction with RBCs and plasma, resembling the clinical situation of a transfused patient more closely, is also taken into account in our study. Multiple *in vitro* and *in vivo* studies have indicated that RBCs and

plasma affect platelet function and hemostasis.[14-18] When stored platelets are *ex vivo* mixed with whole blood, the agonist-induced CD62P expression and aggregation improve,[19, 20] which implies the influence of RBCs and/or plasma on platelet function. Even while the PAS-C-platelets and plasma-platelets were mixed with RBCs and plasma, in our experiment, which may partially recover platelet function, we still observed noticeable differences.

Both Multiplate and TEG are whole blood assays, so during the measurement all plasmatic and cellular components of blood are present. TEG is intended to measure the overall hemostasis, while Multiplate aims to measure only the platelet aggregation. In multiple clinical studies, both Multiplate and TEG have been proven to identify clinically relevant thrombocytopathies and coagulopathies, respectively. Both techniques seem to have added value in guiding transfusion policy in several clinical settings.[11, 21-23] Agonist-induced CD62P expression is correlated to bleeding outcome in ITP patients [24] also suggesting that our results may be clinically relevant. Further research is needed to verify whether the observed differences between PAS and plasma stored platelets are also found *in vivo*, as also vessel walls, blood flow and other patient factors affect clot formation.

The whole blood**,** reconstituted with PAS-C-platelets had a platelet concentration and hematocrit which were not different from those of the plasma-platelet-samples, and are therefore not expected to affect Multiplate and TEG results.[25, 26] We found that the aggregation capacity of PAS-C-platelets was lower than of plasma-platelets. Also, the agonist-induced CD62P responsiveness of PAS-C-platelets was significantly lower than the responsiveness of plasma-platelets. These results show that besides the dilution of coagulation factors by the PAS-fluid, also the platelet function itself may be a concern for the hemostatic function of PAS-platelets. This study is to our knowledge the first to demonstrate a difference in platelet function using Multiplate and CD62P responsiveness between PAS-platelets and plasma-platelets. However, the TEG-derived parameters showed no differences between reconstituted whole blood with PAS-C-platelets and reconstituted whole blood with plasma-platelets. These results suggest that the differences between the platelet types in agonist-induced aggregation and CD62P responsiveness do not result in a difference in overall clot formation. The explanation for these TEG findings could be that the platelets are activated via other pathways than ADP and TRAP, which were affected in the Multiplate, and that although the "responsiveness" is diminished, it does not affect overall clot formation.

With increasing storage time there was a reduction in aggregation capacity for both platelet types with all agonists. These results are in agreement with those of others.[19, 27, 28] In addition, our findings show that the difference between the aggregation

capacity of plasma-platelets and PAS-C-platelets exists despite of the addition of RBCs and plasma and remains with increasing storage time. The clot formation capacity of the samples showed minimal changes over storage time, which is in agreement with an earlier study analyzing TEG parameters of platelet concentrates in the absence of RBCs and plasma.[27] We can only speculate that this is indicative of an actual platelet transfusion,[20] showing that after transfusion the platelet quality remains affected despite of the interaction with RBCs and plasma. More studies are needed in this respect. In our study, a storage time of 13 days was analyzed, but platelets stored more than 7 days are not transfused in clinical practice. The 13-day storage time shows that the pattern of the first 7 days is consistent with the downward trend of the subsequent storage days.

The results of our experiment show that both the aggregation and the CD62P responsiveness of PAS-C-platelets were lower than those of plasma-platelets. Moreover, transfusion of substantial amounts of PAS-C-fluid may aggravate the impairment of hemostasis, as the additional experiment suggests. It should be noted that in our experiment the plasma fraction of the reconstituted whole blood was completely replaced by PAS-C-supernatant, 65% PAS and 35% plasma, which is unlikely to occur to this extent in clinical practice. In the situation of a massive transfusion also other (blood) products are administered and the platelet products that are transfused can have various storage times. Therefore, the difference observed in this study is larger than expected in clinical practice. Nevertheless, clinicians should be aware of the potential impairment of platelet function and hemostasis with massive transfusion of PAS-C-platelets and accompanying PAS-C-fluid. This is in agreement with the recommendations, based on the results of the PROPPR-trial, to avoid PAS-platelets in resuscitation of trauma patients with massive bleeding.[29] In conclusion, our results demonstrate that the function of PAS-C-platelets is inferior to that of plasma-platelets in reconstituted whole blood. These results may have implications in the setting of massive transfusions.

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CHAPTER 4

Effect of storage of platelet concentrates in PAS-B, PAS-C or plasma on transfusion reactions

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Background

Reports on the clinical consequences of longer storage time of platelet concentrates are contradictory. The objective of this study was to assess whether longer storage times are associated with a higher risk of transfusion reactions.

Study design and methods

We gathered storage times of pooled platelet concentrates related to transfusion reactions reported to the national hemovigilance office from 2004 to 2015. These were combined with storage times of platelet concentrates in the reference population to compare incidences of transfusion-associated circulatory overload, transfusion-related acute lung injury, allergic reactions, febrile non-hemolytic reactions, and "other" reactions between storage time categories.

Results

A total of 567,053 platelet concentrates and 1,870 transfusion reactions were analyzed. Among platket additive solution (PAS)-B-platelet recipients, the odds ratio of a storage time of 4 to 5 days compared to 1 to 3 days was 1.60 (95% confidence interval (CI) 1.17;2.18) for allergic, and 1.47 (95%CI 1.09;1.98) for febrile reactions. For PAS-C-platelet recipients, the odds ratio for allergic reactions was 3.78 (95%CI 1.31;10.9) for 4 to 5 days, and 4.57 (95% CI 1.57;13.4) for 6 to 7 days old platelets when compared to 1 to 3 days old units. In all other studied reaction types no statistically significant association was observed in platelets in plasma, PAS-B and PAS-C.

Conclusions

In plasma-platelets longer storage time was not associated with a higher incidence of transfusion reactions. In PAS-platelets longer storage time was associated with higher transfusion reaction incidences, in particular for allergic reactions with both PAS-fluids and febrile reactions with PAS-B. This indicates that the effect of storage time is different for different reaction types and depends on the storage fluid.

Abstract

Introduction

Platelet transfusions are used to provide hemostatic capacity in patients with a decreased number or functionality of platelets.[1] However, platelet transfusions can cause adverse events as well, such as allergic reactions, febrile non-hemolytic reactions, transfusion-associated circulatory overload, transfusion-related acute lung injury (TRALI) and other reactions. All transfusion reactions cause some degree of inconvenience for patients and involve increased costs, and may result in (severe) morbidity, or even mortality.[2,3] Therefore, over the past few decades extensive effort has been made to identify the factors contributing to the occurrence of transfusion reactions. Multiple factors, such as storage fluid, leukoreduction, collection method (apheresis or pooled buffy coats), and storage time have been studied for their roles in inducing transfusion reactions after platelet transfusions.[2,4,5] As a result of these earlier findings multiple improvements, such as leukoreduction, have been achieved in the production of platelet concentrates.[6,7] Nevertheless, platelet transfusions are still associated with relatively high incidences of transfusions reactions.

Storage time has been associated with the accumulation of biological response modifiers, such as inflammatory cytokines and chemokines.[8-11] Whether these changes also have clinical consequences is not clear yet, as published results are contradictory.[5,12] A recently published review paper concludes that the risk of transfusion reactions was similar in old, compared to fresh, leukoreduced units,[13] whereas a more recent study, not included in the review, showed that prolonged storage of platelets was associated with a higher frequency of inflammatory transfusion reactions, but not allergic reactions.[12]

This controversy indicates that a better understanding of the influence of storage time on the development of transfusion reactions is warranted, and will create the opportunity to further improve transfusion safety.[2,14-17] Therefore, the objective of this study was to assess the association of storage time of leuko-reduced buffy-coat platelets stored in plasma, platelet additive solution (PAS) B, or PAS C with the incidence of allergic reactions, febrile non-hemolytic reactions, transfusion-associated circulatory overload, TRALI and "other reactions".

Material and methods

Study design

This nationwide, case-referent study evaluated the impact of storage time of platelets on transfusion reaction rates in the period from 2004 to 2015.[18] Anonymized data, further described below, were obtained from the national hemovigilance organization

'Transfusion and Transplantation Reactions in Patients' (TRIP) and the national Sanquin database (ePrognosa, MAKsystems, Paris, France). The reference distribution of the storage time of all transfused platelet units was estimated using a database, as described earlier, containing data of more than 100,000 pooled platelet units transfused in the Netherlands.[19]

Platelet products and storage time

All platelet products, pre-storage leuko-reduced by filtration, were produced and stored by Sanquin Blood Bank (Amsterdam, the Netherlands). About 90% of platelet concentrates were prepared using five ABO identical and Rh-D-compatible buffy coats from whole blood donations. These five buffy coats, each containing up to 30 mL of plasma, were re-suspended either in plasma of one of the five donors (plasma-platelets; January 1, 2006 to December 31, 2015) or in PAS (PAS-platelets). Two types of PAS were used during the studied period: PAS-B (also known as PAS-2 or T-Sol, Baxter (Nivelles, France), January 1, 2004 to November 30, 2012) and PAS-C (also known as PAS-III or Intersol, Fenwal, a Fresenius company (La Châtre, France) December 1, 2012 to December 31, 2015). The remaining 10% of platelet units were collected by apheresis. Apheresis and hyper-concentrated platelet units were excluded from this analysis, as these are transfused for specific indications, including previous transfusion reactions. The maximum storage time was 7 days for plasma- and PAS-C-platelets and 5 days for PAS-B-platelets. The total numbers of distributed units per type of platelet product over the studied periods in the Netherlands were obtained from the national Sanquin database (ePrognosa). The storage time of the platelet units involved in reactions was calculated by subtracting the date of the blood donation, based on the ePrognosa database, from the date of transfusion, based on the TRIP database. The storage times were classified in the three categories 1 to 3 days, 4 to 5 days and 6 to 7 days.

Hemovigilance system

All data on the transfusion reactions were obtained from TRIP. TRIP is the hemovigilance system in the Netherlands that has been operational since 2003.[20] Participation of a hospital is regarded as the professional standard both in the national transfusion guideline and by the Healthcare Inspectorate.[21] Since 2008, in accordance with European legislation, the reporting of serious reactions to TRIP, in parallel to the Healthcare Inspectorate as the competent authority, has been mandatory. Participation by the hospitals has been over 90% each year from 2004.[20] We evaluated transfusion reactions, reported between January 1, 2004, and December 31, 2015 for which the storage time of the platelet units could be determined. The definitions of reportable reaction types have been published previously.[20] These are mostly similar to the international definitions developed by the International Hemovigilance Network in collaboration with the International Society for Blood Transfusion.[22] In these definitions, febrile non-hemolytic

transfusion reactions (FNHTRs) and mild FNHTRs are recorded separately, with FNHTRs being characterized by a temperature rise of ≥ 2°C and/or rigors, and mild FNHTRs by a temperature rise ≥1 and <2°C without rigors. Mild FNHTRs were excluded from our analyses, because not all hospitals report mild FNHTRs to TRIP. The reaction type "allergic reaction" included both anaphylactic reactions and other allergic reactions. (Suspected) bacterial and viral transfusion transmitted infections were excluded as these have been reported elsewhere.[19] The reaction type "other reaction" is a collection of reactions that do not fit in the definition of one of the reaction categories defined by TRIP. Furthermore, only reactions with imputability "certain", "probable" or "possible" were included in our analyses. If multiple units of platelets were associated with a reaction, the reaction was included only if the units were in the same storage time category.

Statistical analyses

The association of storage time with transfusion reaction incidences was assessed separately for each of the three platelet storage media (PAS-B, PAS-C and plasma), because the maximal storage period and thereby the distribution of the storage times differs. Moreover, differences are observed between the storage media regarding the associated transfusion reaction incidences. Most importantly, there might also be effect modification by the storage medium. The effect of storage time on transfusion reaction incidences was evaluated using logistic regression derived odds ratios with 95% confidence intervals (CIs). Hospital could be a confounder in our analyses, because hospital is correlated both with the storage time distribution and with the reported transfusion reaction rate. The distribution of storage time of transfused blood products might differ between hospitals because of differences in location, scale, patient population and policy. The rate of reported transfusion reactions of hospitals may be influenced by transfusion policy and practice, patient population, and hospital reporting instructions as well as culture. To correct for confounding by hospital, weighting was performed based on the hospital's reporting tendency. As all Dutch hospitals use the same type of red blood cell units, the incidence of reported transfusion reactions following RBC transfusions was used as a measure for the reporting tendency of the hospital. For university hospitals the incidences were calculated separately for every hospital. As in several non-university hospitals the incidence of RBC transfusion reactions is very low and often close to zero, which would give unstable and unrealistic weights, the weight for the non-university hospitals was calculated for the whole group of non-university hospitals. All incidences of the hospital (group) were divided by the national incidence, and then the inverse of this ratio was used as a weight. The transfusion reactions were weighted by these calculated inverses in our analyses. This can be summarized in the following equation:

$$\textit{weighted incidence per hospital} = \frac{\textit{unweighted incidence per hospital}}{\textit{(hospital RBC transfusion reaction incidence / national RBCs transfusion reaction incidence)}}$$

Results

Population

A total of 567,053 pooled platelet units and 1,870 transfusion reactions were analyzed in our study (shown in table 1). The majority of the 1,870 transfusion reactions, 1081 (57.8%), were allergic reactions, 547 (29.3%) were febrile reactions, 39 (2.1%) were cases of transfusion-associated circulatory overload, 24 (1.3%) were cases of transfusion-related acute lung injury (TRALI), and 179 (9.6%) were classified as "other reaction". From 2006 to 2015 a total of 425,127 plasma-platelet units were distributed and during this period 1,472 transfusion reactions were reported to TRIP that followed transfusion of plasma-platelets. In the period from 2004 to 2012 a total of 96,669 PAS-B-platelet units were distributed, and 297 reactions were reported that were associated with PAS-B-platelet transfusions. From 2012 to 2015, 45,227 units of PAS-C-platelets were distributed, and 101 reactions associated with PAS-C-platelet transfusions were reported. The crude, unweighted incidences per storage fluid per storage time category and overall incidences are reported in the supporting information.

Storage time and transfusion reactions

For plasma-platelets the odds ratios per reaction type per storage time category compared to the reference category are shown in table 2. In patients receiving plasma-platelets no differences were observed between older and fresher units for any of the specified reactions. In PAS-B platelets, shown in table 2, the transfusions with older (4 to 5 day old) units were associated with more allergic reactions compared to the fresher (1 to 3 day old) units, odds ratio 1.60 (95%CI 1.17;2.18). Also, the older PAS-B-platelets were associated with more FNHTRs than the fresher units, odds ratio 1.47 (95%CI 1.09;1.98). No statistically significant differences were observed between older and fresher units in PAS-B for the other reaction types. In PAS-C-platelets transfusions with older units were also associated with more allergic reactions (table 2). Units with a storage time of 4 to 5 days (odds ratio 3.78, 95%CI 1.31;10.9), as well as units of 6 to 7 days, odds ratio (4.57, 95%CI 1.57;13.3), were associated with more allergic reactions compared to the reference group. No statistically significant differences were observed between older and fresher PAS-C units for the other reaction types. The odds ratios found in the crude unweighted analysis were comparable to the results of the weighted analysis (supporting information).

Table 1 Crude number of distributed platelet concentrates and reported transfusion reactions per storage fluid

	Storage fluid		
	plasma	**PAS-B**	**PAS-C**
Transfusions			
Total number of transfusions	425,127	96,699	45,227
Mean storage time (days)	4.74	3.45	4.50
Median (IQR) storage time (days)	5 (3 - 6)	3 (2 - 4)	4 (3 - 6)
Reactions			
Total number	1,472	297	101
Per storage time category			
- 1 - 3 days	387	120	21
- 4 - 5 days	512	177	37
- 6 - 7 days	573		43
Mean storage time (days)	4.78	3.69	5.04
Median (IQR*) storage time (days)	5 (3 - 6)	4 (3 - 5)	5 (4 - 6)
Years included	2006 - 2015	2004 - 2012	2012 - 2015

* IQR interquartile range

Discussion

In this nationwide study, older PAS-B-platelets were associated with a higher incidence of allergic and febrile reactions. Among PAS-C-platelets, the older units were associated with a higher allergic reaction incidence compared to fresh units. In plasma-platelets, no statistically significant differences were observed between fresher and older units with regard to any of the transfusion reactions.

Strengths and limitations

An important strength of our study is that it spans a period of 10 years and is nationwide, which means that it covers all patients who were transfused with pooled leukoreduced platelets. Moreover, our analysis included over 500,000 platelet transfusions, which made it possible to analyze the different platelet products and reaction types separately. Furthermore, in our study it was possible to estimate the effect of storage time on transfusion reactions with great precision. A limitation of our study was that the distribution of the storage time of all transfused platelet units was estimated based on a subset of the Dutch hospitals. It is possible that this dataset is not completely representative of the source population. However, data of more than 100,000 transfusions are included of both university and large general hospitals located in different regions of the Netherlands, so large, systematic deviations from the source population

Table 2 Odds ratios (with 95% CI) for transfusion reactions after platelet concentrate transfusion: old versus fresh units

Plasma-platelets	**Storage time**		
Reaction type	**1 - 3 days**	**4 - 5 days**	**6 - 7 days**
Allergic reaction	reference	0.95 (0.81;1.13)	1.02 (0.87;1.21)
FNHTR	reference	1.07 (0.82;1.39)	1.15 (0.89;1.49)
TRALI	reference	1.56 (0.50;4.80)	1.44 (0.46;4.52)
TACO	reference	0.45 (0.18;1.15)	0.78 (0.35;1.75)
Other reaction	reference	0.82 (0.53;1.28)	1.13 (0.75;1.71)
Overall	reference	0.96 (0.84;1.10)	1.06 (0.93;1.21)
PAS-B-platelets	**Storage time**		
Reaction type	**1 - 3 days**	**4 - 5 days**	
Allergic reaction	reference	1.60 (1.17;2.18)	
FNHTR	reference	1.47 (1.09;1.98)	
TRALI	reference	NA	
TACO	reference	1.56 (0.43;5.67)	
Other reaction	reference	1.44 (0.69;3.02)	
Overall	reference	1.54 (1.26;1.89)	
PAS-C-platelets	**Storage time**		
Reaction type	**1 - 3 days**	**4 - 5 days**	**6 - 7 days**
Allergic reaction	reference	3.78 (1.31;10.9)	4.57 (1.57;13.3)
FNHTR	reference	0.90 (0.37;2.19)	1.93 (0.86;4.31)
TRALI	reference	NA	NA
TACO	reference	0.56 (0.04;7.31)	NA
Other reaction	reference	0.60 (0.16;2.24)	0.73 (0.19;2.77)
Overall	reference	1.41 (0.81;2.44)	2.03 (1.18;3.49)

Odds ratios with 95% confidence interval, weighted for reporting rate, with storage time of 1 to 3 days as the reference category.

FNHTR non-hemolytic transfusion reaction; NA not applicable; TACO transfusion-associated circulatory overload; TRALI transfusion-related acute lung injury

are unlikely. Also, not all transfusion reactions could be included in the analysis as not all storage times could be determined. However, we do not believe that this poses a considerable problem as we do not expect an association between the storage time of a product and the chance that the storage time could be determined. In our database, no patient identifier was available, so it was impossible to correct for the potential influence of patient level dependency. However, it is unlikely that this had a significant influence on the studied association as there is no reason to assume that both storage time and transfusion reactions cluster at patient level. The TRIP data are based on passive surveillance of transfusion reactions. This is both a limitation, because not all reactions

are detected and reported, and a strength, because the reactions that are reported are probably the most relevant reactions.

Plasma-platelets

It has been demonstrated that older platelets show *in vitro* deterioration and in transfused patients result in inferior laboratory measurements (like corrected count increments) compared to fresher platelets.[23,24] However, the clinical impact on patient outcomes, like transfusion reactions, is not clear yet. A recent meta-analysis summarizing the effect of storage time on clinical outcomes concluded that older platelet products were associated with more transfusion reactions.[5] However, the increased risk of reactions was not observed when leuko-reduced products were analyzed separately, which is in agreement with our findings in plasma-platelets. As our study contains a considerably larger sample size, our findings strengthen the plausibility of the earlier findings for plasma-platelets.[25,26]

Another recent study showed that prolonged storage of plasma-platelets was associated with more inflammatory transfusion reactions (including FNHTRs, TRALI, transfusion associated dyspnea and atypical reactions), but not with allergic reactions.[12] Regarding allergic reactions this other study is in agreement with our study. However, the association in this study between storage time and inflammatory reactions was not confirmed in our study. In the other study not only storage time, but also irradiation and the collection method apheresis were strongly associated with inflammatory reactions. The finding that apheresis as collection method is associated with more transfusion reactions than pooled leuko-reduced platelets is affirmed by others.[2] The fact that apheresis seems to increase the incidence of transfusion reactions, may explain that this other study found an association between storage time and inflammatory reactions in apheresis plasma-platelets while in our study we found no association in pooled plasma-platelets.

PAS-platelets

The findings of the earlier mentioned meta-analysis are not in agreement with our findings in PAS-platelets. However, the meta-analysis pooled all results irrespective of reaction type; storage fluid; collection method; which storage days were compared and the patient population.

In PAS-B-platelets we found that a longer storage time was associated with a higher incidence of both allergic and febrile reactions. To the best of our knowledge our study is the first clinical study regarding the effect of storage time on allergic and febrile reactions in leuko-reduced PAS-B-platelets.

For PAS-C-platelets we found that storage for 6 to 7 days was associated with a higher overall incidence of transfusion reactions. In the SPRINT trial, both pathogen-reduced and conventional apheresis platelet concentrates were analysed. Based on these data, a odds ratio of 2.36 (95% CI 1.33;4.19) was calculated in the previously described meta-analysis for 4 to 5 days old conventional platelets compared to 1 to 2 days old conventional platelets in PAS-C.[5,27] Although this study reported on apheresis platelets and our study on pooled buffy-coat platelets, these findings are in agreement with each other. Another recent study regarding platelets in PAS-E (SSP+) is less comparable, because only absolute numbers were reported, and because different storage time categories were compared.[28] In a study of platelets in PAS-F (Plasmalyte), a clear association between storage time and transfusion reactions was demonstrated.[29] Not all these platelets were pre-storage leuko-reduced, but the leukoreduction status was one of the factors that was considered in their statistical model so the reported association between storage time and transfusion reactions was calculated independently of the leukoreduction status. Although Plasmalyte is not the same storage fluid as PAS-B and PAS-C, the findings about the effect of storage time on transfusions reactions are in line with our results.

For PAS-B-platelets, storage time was statistically significantly associated with febrile reactions, but for PAS-C-platelets the association was not observed. Only the odds ratio of the oldest category pointed in the same direction, which may be due to a lack of statistical power in the analyses on PAS-C-platelets, but it is also possible that this indicates that platelets in PAS-C are actually more stable during storage.

Clinical implications

In conclusion, in plasma-platelets, storage time is not associated with a higher incidence of transfusion reactions. In PAS-platelets, storage time is associated with higher transfusion reaction incidences, in particular with allergic reactions in both PAS-fluids, and with febrile reactions in PAS-B. Although in plasma-platelets no association was observed between longer storage time and more transfusion reactions, the overall incidence of transfusion reactions following plasma-platelets is still comparable to that of PAS-B-platelets and higher than that of PAS-C-platelets as we showed earlier.[4] Therefore, regarding transfusion reactions, it seems that platelets stored in PAS-C are the best option. However, the fact that the incidence of transfusion reactions increases over storage time may mean that for PAS-C there is also room for improvement.

For clinical practice not only the risk of adverse events like transfusion reactions, but also the haemostatic efficacy of platelet concentrates should be taken into account. Earlier we showed that *in vitro*, in reconstituted whole blood, the function of PAS-C-platelets

seems inferior to the function of plasma-platelets.[30] However, it is not clear whether this affects patient outcomes in clinical practice.

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PART II

Platelet transfusions in cardiac surgery patients

CHAPTER 5

5

Does a platelet transfusion independently affect bleeding and adverse outcomes in cardiac surgery?

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Background

Conflicting results have been reported concerning the effect of platelet transfusion on several outcomes. The aim of this study was to assess the independent effect of a single early intraoperative platelet transfusion on bleeding and adverse outcomes in cardiac surgery patients.

Methods

For this observational study 23,860 cardiac surgery patients were analysed. Patients who received one early (shortly after cardiopulmonary bypass while still in the operating room) platelet transfusion, and no other transfusions, were defined as the intervention group. By matching the intervention group 1:3 to patients who received no early transfusion with most comparable propensity scores, the reference group was identified.

Results

The intervention group comprised 169 patients and the reference group 507. No difference between the groups was observed concerning reinterventions, thromboembolic complications, infections, organ failure, and mortality. However, patients in the intervention group experienced less blood loss and required vasoactive medication 139 of 169 (82%) versus 370 of 507 (74%; odds ratio, 1.65; 95% CI, 1.05 to 2.58), prolonged mechanical ventilation 92 of 169 (54%) versus 226 of 507 (45%; odds ratio, 1.47; 94% CI, 1.03 to 2.11), prolonged intensive care 95 of 169 (56%) versus 240 of 507 (46%; odds ratio, 1.49; 95% CI, 1.04 to 2.12), erythrocytes 75 of 169 (44%) versus 145 of 507 (34%; odds ratio, 1.55; 95% CI, 1.08 to 2.23), plasma 29 of 169 (17%) versus 23 of 507 (7.3%; odds ratio, 2.63; 95% CI, 1.50–4.63), and platelets 72 of 169 (43%) versus 25 of 507 (4.3%; odds ratio, 16.4; 95% CI, 9.3–28.9) more often compared to the reference group.

Conclusions

In this retrospective analysis, cardiac surgery patients receiving platelet transfusion in the operating room experienced less blood loss and more often required vasoactive medication; prolonged ventilation; prolonged intensive care and blood products postoperatively. However, early platelet transfusion was not associated with reinterventions, thromboembolic complications, infections, organ failure, or mortality.

Abstract

Introduction

Patients undergoing cardiac surgery are at increased risk for excessive bleeding. Excessive bleeding may lead to surgical re-exploration. Both excessive blood loss and re-exploration are associated with increased postoperative mortality and morbidity.[1-3] Thus efficient prevention and treatment of the cause of bleeding is an important issue in cardiac surgery. As expected, part of the postoperative bleedings is due to surgically induced injury, but a significant proportion of the observed bleedings can be explained by acquired hemostatic defects.[4,5] Impaired platelet function, mainly due to cardiopulmonary bypass (CPB) and anti-platelet drug therapy, is considered one of the most important hemostatic factors leading to postoperative bleeding.[4-8] Platelet transfusions are thus commonly administered to treat bleeding.[9]

The platelet-transfusion rates vary greatly in cardiac surgery, both nationally and internationally [10], in spite of existing guidelines.[11] This wide variety in platelet transfusion use among cardiac surgery centres, illustrates the lack of consensus on the indication for a platelet transfusion in certain clinical situations. Presumed platelet dysfunction in patients using platelet inhibiting drugs is not always confirmed by a measurement before platelets are transfused. Furthermore, just as in other clinical areas[12,13], there is a lack of clinical evidence establishing the effectiveness of administering platelets in cardiac surgery.[14]

In addition, conflicting results have been reported concerning the effect of platelet transfusions on serious adverse events, like stroke, infections, vasoplegia and death in cardiac surgery.[15-22] The recently published results of a multicenter randomized controlled trial (Platelet Transfusion in Cerebral Haemorrhage (PATCH) trial) comparing standard care to standard care with platelet transfusion in patients using antiplatelet therapy before intracerebral hemorrhage, showed that platelet transfusions seemed inferior to the standard care.[23]

Our hypothesis was that a single early platelet transfusion, in the absence of concomitant erythrocyte or plasma transfusion, is associated with less bleeding complications and is associated with more adverse events, in patients undergoing cardiac surgery.

Patients and methods

Data collection

The analyses were performed using data from the Amphia Cardiac Surgery Registry consisting of 23,860 patients who underwent cardiac surgery at the Amphia Hospital

between 1997 and 2013. Details of this database have been described previously.[24] In this ongoing cohort study, detailed baseline and perioperative data of all consecutive patients undergoing cardiac surgery in the Amphia Hospital were collected. Data collection for the current analysis took place between January 1st 1997 and January 1th 2013 and was compliant with the definitions of the Dutch National Cardiac Surgery Registry, BHN, and the Dutch National Intensive Care Registry, NICE (instituted in 1996).[25] All patient-care decisions were taken by the attending physician in accordance with transfusion and coagulation hospital guideline based protocols. Members of our departmental review committee critically reviewed the analytical plan. The aim of the study, the inclusion and exclusion criteria, propensity score matching as the method to correct for confounding by indication, the postoperative endpoints, and logistic regression as the method to analyse the endpoints were determined before examination of the data. There was no *a priori* statistical power analysis calculation used to guide sample size. Sample size and analyses were based on the available data. The ratio and caliper of the propensity score matching were determined during examination of the data. An acknowledged Dutch medical ethical committee approved this study protocol and waived individual patient consent.

Patient sample

It was decided in advance to select only patients who received one early platelet transfusion, defined as one platelet transfusion after the end of cardiopulmonary bypass (CPB) while still in the operating room. Patients transfused with more than one unit of platelets were excluded presuming that these patients would not be comparable to patients who were not transfused with platelets. Also patients who received other blood products in the operating room were excluded aiming at studying the independent effect of an early platelet transfusion without the potential influence of erythrocyte or fresh frozen plasma (FFP) transfusions.

The platelet units transfused in this study consisted of five pooled buffy coats and contained approximately 300 * 10^9 platelets suspended in plasma with or without platelet additive solution. Since 2001 all platelet units were pre-storage leukocyte-reduced in The Netherlands, and before 2001, platelet units were leukocyte-reduced when indicated in the Amphia Hospital. The decision to transfuse platelets was made according to a cardiac surgery coagulation algorithm, with upscaling treatment modalities in which platelet transfusion is used as a last resort after considering other pharmacological strategies. Some degree of freedom was left to the discretion of the physicians, but platelet count lower than 50 * 10^9/l was an indication for platelet transfusion in any case. When platelet count was lower than 100 * 10^9/l and bleeding was present, this was also indication for

transfusion of platelets. No specific platelet function test was available, but in recent years rotational thromboelastometry was included in the algorithm.

Patients who received an early platelet transfusion may differ in various ways from patients who received no early transfusion, because there was a reason to administer the platelet unit (confounding by indication). To correct for this confounding we estimated a propensity score for these patients, representing the probability that the patient received platelets conditional on relevant covariates before the decision to transfuse platelets. The patients who received one early platelet transfusion and no other blood products, and were suitable for propensity score matching were defined as the intervention group. The intervention group was then matched to the reference group, consisting of patients who received no early transfusion and had the closest propensity scores. For the propensity score matching we used the "psmatch2" function in Stata Statistical Software (Release 14; StataCorp LP, College Station, TX, USA) as "greedy" in random order, nearest neighbour 1:3 matching with replacement. Only controls with a propensity score within 0.01 distance (caliper) of the propensity score of the case were selected.[26] We hereby aimed at selecting an intervention and reference group with comparable baseline characteristics. We excluded patients in whom an intraoperative circulatory arrest was part of the surgical procedure because of their exceptional hemodynamic and hemostatic state. Furthermore, Jehovah's Witnesses were excluded as they may be treated with different surgical and anesthesiologic strategies.

Postoperative outcomes

As a result of numerous previous articles reporting contradictory results about the effect of platelet transfusion in cardiac surgery patients, the aim of our study was to obtain an overall picture of all potential consequences for a clinician who is considering an early platelet transfusion for a cardiac surgery patient. So before initiation of the analysis of this study we defined the outcomes we were interested in (based on previous literature and clinical knowledge). Our objective was to study not only the intended effects of an early platelet transfusion (preventing / treating bleeding complications), but also the possible adverse events associated with a platelet transfusion. We aimed at analysing all relevant factors, so both the potential beneficial effects, and the potential undesired effects. We planned to study the following postoperative outcomes: amount of blood loss within 12 hours; early re-exploration for bleeding and/or tamponade; late intervention for tamponade; stroke; myocardial infarction (MI); infections; systemic inflammatory response syndrome; shock; acute kidney injury; multi organ failure; in-hospital mortality; composite endpoint (consisting of MI, stroke, acute kidney injury and in-hospital mortality). Definitions of postoperative MI, acute kidney failure, stroke were described previously.[21] Infection was categorized as pneumonia, mediastinitis, sepsis and others infections with the diagnoses requiring organisms isolated from culture(s) in combina-

tion with elevated temperature and leukocyte counts. Systemic inflammatory response syndrome was diagnosed if two or more of the following criteria were present: temperature > 38 or < 36 degree Celsius; tachypnea (> 20/minute) or hypocapnea (pCO_2 < 4,4 kPa / 32 mmHg); tachycardia (> 90 bpm) or need of mechanical ventilation and leukocyte count > 12 or < 4 * 10^9/l. Multi organ failure was defined as simultaneous or sequential dysfunction or failure of two or more organ systems. Shock was defined as a syndrome in which the effective capillary and tissue perfusion declined to a level detrimental to cellular metabolism. Also we compared duration of postoperative mechanical ventilation and intensive care unit (ICU) stay (both in hours); requirement of postoperative inotropic or vasoactive drugs and erythrocyte, FFP and platelet transfusions in the ICU. Amount of blood loss, duration of mechanical ventilation and ICU stay were analysed as being high or low, with the median as the cut-off point.

Statistical analysis

The continuous baseline variables were summarized by medians and interquartile ranges and the categorical variables were summarized by frequencies and percentages. The propensity score was generated with logistic regression and the variables, where the propensity score was based on, were chosen based on previous knowledge of the subject, as suggested in previous articles.[27-29] The following preoperative variables were included in the propensity score: age, gender, year of surgical procedure (per calendar year), previous cardiac surgery, history of MI, acetylicsalicylic acid or clopidogrel use, (continued up to surgery, stopped preoperatively, or never used), known vascular disease, chronic obstructive pulmonary disease, diabetes, atrial fibrillation, angina pectoris, active endocarditis, hemoglobin level, international normalized ratio, acute or chronic renal failure, left ventricular ejection fraction, immunosuppressant drug use, type of surgery, nonelective surgery, cardiopulmonary resuscitation within 24 h before surgery, respiratory insufficiency, off-pump surgery, CPB duration, and European System for Cardiac Operative Risk Evaluation (EuroScore). It was not in all years part of the standard care to determine fibrinogen level and platelet function before surgery and/or transfusion, so these measures were not available for analysis. Missing variables were imputed using single imputation strategies. For the propensity score matching, we used the "psmatch2" function in Stata Statistical Software (Release 14; StataCorp LP, USA), nearest neighbor 1:3 matching with replacement. Only controls with a propensity score within 0.01 distance (caliper) of the propensity score of the case were selected.29 To assess the balance in measured baseline characteristics after propensity score matching between treated and untreated patients, the standardized mean differences were determined. The matching procedure was optimized based on observed balance in baseline variables before examination of the outcome results. Comparisons of outcomes were made between the intervention and reference groups with regard to odds ratios with 95% CIs derived from multiple univariate logistic regression analyses. Given the fact

that 1:3 matching with replacement was applied, the clustered pattern of the data was taken into account in the estimation procedure by using a robust (sandwich) estimator in the logistic regressions, specifying the patient identifying number. Additionally, we performed two sensitivity analyses. First, we corrected the logistic models for baseline characteristics that remained unbalanced after the matching procedure. Second, we corrected the logistic regressions for a baseline characteristic with a standardized difference below 10% because of its high clinical relevance. No adjustments were made for testing multiple outcomes.

Results

Patient characteristics

The database comprised 23,860 patients in total, of whom 17,918 remained after application of the exclusion criteria (figure 1). Several of the relevant baseline characteristics of the 171 patients who received an early platelet transfusion were evidently different from the ones of the 17,747 patients who received no early transfusions (shown on the left side of table 1). By propensity score matching the patients were selected from the 17,747 patients who received no early transfusion and were most comparable with the patients who received an early platelet transfusion. Of the 171 patients who received one early platelet transfusion, 169 patients had propensity scores overlapping with the propensity scores of the patients who received no early transfusions (*i.e.* had the same "baseline risk" of receiving a platelet transfusion). So these 169 patients were suitable for propensity score matching and thereby formed the intervention group. The reference group, which was formed after 1:3 propensity score matching, consisted of 507 patients (who had not received any blood product in the operating room). Considering the fact that matching with replacement was

used control patients could be used multiple times: 444 controls were used once, 24 controls were used twice, and 5 were used three times, summing up to 473 unique controls out of 507 controls in total.

The majority of patients were men (81%), the median age was 67 years and about half the patients (49%) had a history of myocardial infarction. Most patients underwent isolated coronary artery bypass graft (CABG) (69%) and almost one quarter (23%) of all procedures was a nonelective procedure. As expected, the balance of multiple clinically important variables improved after propensity score matching. Among others the balance of gender, year of surgery, history of MI, clopidogrel use, type of surgery, EuroSCORE, nonelective surgery, CPR within 24 h before surgery and CPB time improved remarkably (shown on the right side of table 1). Standardized differences for

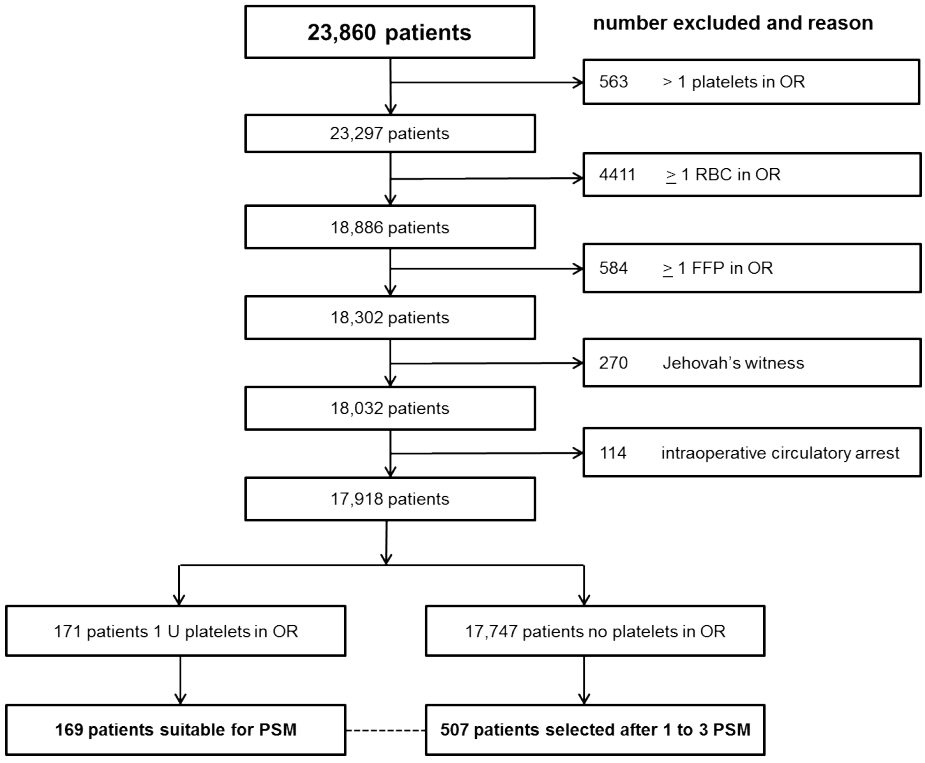

Figure 1 Flow chart of selection of the intervention and reference group

FFP fresh frozen plasma
OR operating room
PSM propensity score matching
RBC red blood cell

the baseline characteristics are reported for the unmatched and matched groups. Two of the measured covariates, "cellsaver blood returned or not" and "nadir intraoperative hemoglobin," had standardized differences that slightly exceeded 10%, indicative of imbalance in these covariates between matched-treated and untreated patients. The second sensitivity analysis was corrected not only for variables with standardized differences above 10%, but also for a variable with better balance, but with high clinical relevance, namely, the EuroSCORE.

Early platelet transfusion and outcomes

Patients in the intervention group less often experienced blood loss higher than 500 ml than patients in the reference group (odds ratio, 0.66; 95% CI, 0.46 to 0.94). However, the number of early re-explorations for bleeding and / or tamponade and number of late interventions for tamponade of patients in the intervention group did not significantly

Table 1 Patient characteristics before and after propensity score matching

	Before propensity score matching			After propensity score matching		
	1 early platelet transfusion n=171	no early transfusion n=17,747	SMD (%)	Intervention group n=169	Reference group n=507	SMD (%)
Preoperative variables						
Female sex*	33 (19.3)	4107 (23.1)	9.4	32 (18.9)	97 (19.1)	0.5
Age (year)*	67 (61-74)	66 (59-73)	0.5	67 (60-73)	67 (58-73)	4.3
Weight (kg)	81 (75-90)	80 (72-90)	10.2	81 (75-90)	80 (73-90)	9.9
Year of surgery*	2003 (1998-2008)	2004 (2001-2009)	26.5	2003 (1998-2008)	2003 (1999-2007)	2.5
Previous cardiac surgery*	21 (12.3)	1290 (7.3)	16.9	21 (12.4)	56 (13.2)	2.7
History of MI*	83 (48.5)	6441 (36.3)	24.9	81 (47.9)	253 (49.9)	4.0
Affected coronary arteries*	3 (1-3)	3 (1-3)	7.9	3 (1-3)	3 (1-3)	4.3
LV hypertrophy*	50 (29.2)	3623 (20.4)	20.5	50 (29.6)	166 (32.7)	7.3
LMCA occluded >50%	30 (17.5)	2661 (15.0)	6.9	29 (17.2)	95 (18.7)	4.3
Acetylsalicylic acid use*						
• Continued up to surgery	21 (12.3)	1133 (6.4)	20.3	20 (11.8)	60 (11.8)	0.0
• Stopped before surgery	81 (47.4)	9181 (51.7)	8.7	81 (47.9)	253 (49.9)	3.9
• Never	69 (40.4)	7433 (41.9)	3.1	68 (40.2)	194 (38.3)	4.0
Clopidogrel use*						
• Continued up to surgery	20 (11.7)	362 (2.0)	38.8	19 (11.2)	59 (11.6)	1.6
• Stopped before surgery	32 (18.7)	1974 (11.1)	21.4	31 (18.3)	101 (19.9)	4.4
• Never	119 (69.6)	15411 (86.8)	42.6	119 (70.4)	347 (68.4)	4.9
Hypertension	85 (49.7)	9443 (53.2)	7.0	84 (49.7)	251 (49.5)	0.4
Hypercholesteremia	105 (61.4)	11583 (65.3)	8.0	105 (62.1)	325 (64.1)	4.1
Smoking	29 (17.0)	3560 (20.1)	8.0	28 (16.6)	98 (19.3)	7.1
Vascular disease*	26 (15.2)	2558 (14.4)	2.2	25 (14.8)	76 (15.0)	0.6
COPD*	21 (12.3)	2485 (14.0)	5.1	20 (11.8)	53 (10.5)	4.1
Diabetes mellitus*						
Diabetes mellitus I	3 (1.8)	545 (3.1)	8.6	3 (1.8)	7 (1.4)	2.6
Diabetes mellitus II	23 (13.5)	2617 (14.7)	3.7	23 (13.6)	70 (13.8)	0.6
Atrial fibrillation*	20 (11.7)	2404 (13.5)	5.6	20 (11.8)	54 (10.7)	3.6
Endocarditis*	3 (1.8)	84 (0.5)	12.2	3 (1.8)	9 (1.8)	0.0
Hemoglobin (g/dL)*	14.0 (13.2-15.0)	14.2 (13.2-15.0)	9.0	14.0 (13.2-15.0)	14.2 (13.0-15.0)	0.7
APTT (s)	34 (28-40)	32 (28-38)	23.5	34 (28-40)	33 (28-40)	4.0
INR*						
• < 1.5	122 (71.3)	13620 (76.7)	12.3	122 (72.2)	356 (70.2)	4.5
• 1.5 – 2.5	38 (22.2)	3472 (19.6)	6.5	37 (21.9)	120 (23.7)	4.4
• > 2.5	11 (6.4)	655 (3.7)	12.5	10 (5.9)	31 (6.1)	0.9
Creatinine (µmol/L)	88 (76-101)	86 (75-99)	7.2	88 (76-101)	88 (77-103)	1.8
Chronic renal failure *	2 (1.2)	218 (1.2)	0.5	2 (1.2)	2 (0.4)	7.2
Acute renal failure*	4 (2.3)	130 (0.7)	13.1	4 (2.4)	12 (2.4)	0.0

Table 1 continued

	Before propensity score matching			After propensity score matching		
	1 early platelet transfusion n=171	**no early transfusion n=17,747**	**SMD (%)**	**Intervention group n=169**	**Reference group n=507**	**SMD (%)**
LV ejection fraction*						
• >50%	110 (64.3)	13386 (75.4)	24.3	108 (63.9)	344 (67.9)	8.6
• 25-50%	42 (24.6)	2813 (15.9)	21.7	42 (24.9)	107 (21.1)	9.4
• < 25%	19 (11.1)	1548 (8.7)	8.0	19 (11.2)	56 (11.1)	0.7
Immunosuppressive drugs*	8 (4.7)	604 (3.4)	6.5	7 (4.1)	25 (4.9)	4.0
Tricuspid valve pathology	6 (3.5)	476 (2.7)	4.8	6 (3.6)	16 (3.2)	2.3
Mitral valve pathology	33 (19.3)	2449 (13.8)	14.8	32 (18.9)	88 (17.4)	4.3
Aortic valve pathology	42 (24.6)	3885 (21.9)	6.3	42 (24.9)	115 (22.7)	5.1
Type of surgery*						
• Isolated CABG	115 (67.3)	12532 (70.6)	7.3	114 (67.5)	351 (69.2)	3.8
• Other than isolated CABG	56 (32.7)	5215 (29.4)	7.3	55 (32.5)	156 (30.8)	3.8
EuroSCORE I*	6 (3-9)	4 (2-7)	50.5	6 (3-9)	6 (3-9)	6.7
NYHA class IV*	35 (20.5)	2871 (16.2)	11.1	34 (20.1)	88 (17.4)	7.1
Non-elective surgery*	42 (24.6)	1089 (6.1)	52.8	41 (24.3)	114 (22.5)	5.1
CPR in 24 h before surgery*	6 (3.5)	128 (0.7)	19.4	6 (3.6)	16 (3.2)	2.7
Intra-operative variables						
Surgical procedure time (min)	251 (208-310)	233 (196-275)	30.0	251 (208-307)	255 (210-300)	4.4
Aortic occlusion time (min)	69 (47-95)	61 (44-80)	37.0	69 (48-95)	68 (53-90)	0.2
CPB use*	163 (95.3)	15653 (88.2)	26.1	161 (95.3)	488 (96.3)	3.6
CPB time (min)*	106 (78-149)	91 (69-116)	48.8	106 (78-148)	104 (83-136)	3.5
Cellsaver blood given, yes/no	64 (37.4)	6203 (35.0)	5.1	64 (37.9)	166 (32.7)	10.7
Nadir hemoglobin (g/dL)	8.6 (7.7-9.5)	8.7 (7.9-9.7)	11.1	8.6 (7.8-9.5)	8.7 (7.6-9.5)	10.5

* Marks variables that were used to calculate the propensity score.
Continuous variables are reported as median with interquartile range and categorical variables are reported as counts with percentages. Standardized differences are reported in % for assessing balance.
CABG coronary artery bypass graft
CPB cardiopulmonary bypass
CPR cardiopulmonary resuscitation
LMCA left main coronary artery
LV left ventricle
MI myocardial infarction
NYHA New York Heart Association
SMD standardized mean difference

differ from that of patients in the reference group (table 2). Patients in the intervention group did not endure more stroke, MI, infection, systemic inflammatory response syndrome, shock, acute kidney injury, multiorgan failure, death or composite endpoint than patients in the reference group (table 3). An early platelet transfusion was significantly associated with the need for postoperative vasoactive medication (odds ratio 1.65, 95% confidence interval (CI) 1.05 to 2.58); long (above median) mechanical ventilation (odds

Table 2 Postoperative bleeding related outcomes

	Intervention group n=169	Reference group n=507	odds ratio (95%CI)	p-value
Blood loss >500 mL first 12 h	79 (46.7)	290 (57.2)	0.66 (0.46;0.94)	0.021
Early re-exploration for bleeding and/or tamponade	4 (2.4)	23 (4.5)	0.51 (0.17;1.52)	0.227
Late intervention for tamponade	4 (2.4)	6 (1.2)	2.02 (0.52;7.89)	0.309

The absolute numbers and percentages of patients in the intervention and the reference group are given, the regression derived odds ratios with 95% confidence interval and exact p-values.

CI confidence interval

ratio 1.47, 95% CI 1.03 to 2.11) and long (above median) ICU stay (odds ratio 1.49, 95% CI 1.04 to 2.12). Also, patients in the intervention group required erythrocyte (44.4 versus 33.9%), FFP (17.2 versus 7.3%) and platelet transfusion in the ICU (42.6 versus 4.3%) more often as compared to patients in the reference group (table 3).

Discussion

Main findings

In this study, no statistically significant difference was observed with regard to reinterventions for bleeding, stroke, MI, infections, systemic inflammatory response syndrome, shock, acute kidney injury, multiorgan failure, death, or composite endpoint between patients who received a single early platelet concentrate and those who did not. However, patients in the intervention group experienced less blood loss and required postoperative vasoactive medication, long mechanical ventilation, long ICU stay, erythrocyte, FFP, and platelet transfusion in the ICU more often as compared to patients in the reference group.

Interpretation

The observed correlations between early platelet transfusion and less blood loss; longer postoperative mechanical ventilation; longer intensive care stay; and higher rate of administration of vasoactive drugs in the ICU might be explained by a causal effect of the platelet transfusion. For example, the fact that patients in the intervention group experienced less blood loss postoperatively could be due to the perioperative platelet transfusion. In the case of the vasoactive drugs, it may be possible that in the intervention group, more patients suffered from vasoplegia and therefore required vasoactive support more often compared to those in the reference group.. Vasoplegia as the indication, and thus possible causal explanation, of the higher risk of vasoactive

Table 3 Postoperative adverse outcomes

	Intervention group n=169	Reference group n=507	odds ratio (95%CI)	p-value
Stroke*	5 (3.0)	16 (3.2)	0.94 (0.32;2.71)	0.902
Myocardial infarction*	25 (14.8)	57 (11.2)	1.37 (0.82;2.28)	0.226
Patients with postoperative infection	35 (20.7)	88 (17.4)	1.24 (0.79;1.94)	0.340
• Mediastinitis	2 (1.2)	1 (0.2)	6.06 (0.54;67.4)	0.143
• Superficial wound infection	1 (0.6)	2 (0.4)	1.50 (0.14;16.7)	0.740
• Pneumonia	4 (2.4)	13 (2.6)	0.92 (0.30;2.87)	0.887
• Sepsis	11 (6.5)	23 (4.5)	1.47 (0.70;3.08)	0.313
• Other infections	27 (16.0)	62 (12.2)	1.36 (0.82;2.27)	0.230
SIRS	13 (7.7)	25 (4.9)	1.61 (0.79;3.25)	0.187
Shock	28 (16.6)	67 (13.2)	1.30 (0.79;2.14)	0.296
CVVH de novo*	9 (5.3)	19 (3.7)	1.44 (0.63;3.30)	0.383
Multi organ failure	7 (4.1)	16 (3.2)	1.33 (0.53;3.34)	0.549
In hospital mortality*	7 (4.1)	13 (2.6)	1.64 (0.64;4.19)	0.300
Composite endpoint	34 (20.1)	89 (17.6)	1.18 (0.76;1.85)	0.462
Ventilation >11h	92 (54.4)	227 (44.8)	1.47 (1.03;2.11)	0.034
ICU length of stay >26h	95 (56.2)	235 (46.4)	1.49 (1.04;2.12)	0.030
Vasoactive drugs	139 (82.2)	374 (73.8)	1.65 (1.05;2.58)	0.029
RBC transfusion in ICU	75 (44.4)	172 (33.9)	1.55 (1.08;2.23)	0.017
FFP transfusion in ICU	29 (17.2)	37 (7.3)	2.63 (1.50;4.63)	0.001
Platelet transfusion in ICU	72 (42.6)	22 (4.3)	16.4 (9.3;28.9)	<0.001

* Marks endpoints that make up the composite endpoint

The absolute numbers and percentages of patients in the intervention and the reference group are given, the regression derived odds ratios with 95% confidence interval and exact p-values.

CI confidence interval
CVVH continuous veno-venous hemofiltration
FFP fresh frozen plasma
ICU intensive care unit
RBC red blood cell
SIRS systemic inflammatory response syndrome

medication would be in agreement with previous findings of others. First of all it would be consistent with the finding that patients undergoing cardiac surgery with the use of CPB commonly encounter vasoplegia for which pharmacologic support, in the form of vasoactive drugs, is needed.[30,31] More importantly, it would be in agreement with the correlation, demonstrated by others, between platelet transfusion and an increased risk of vasoplegia after cardiac surgery.[16] In our data, the diagnoses shock, SIRS and sepsis, were equally distributed among the groups, and are therefore not a plausible explanation for the difference in vasoactive drug need. This presumed higher rate of vasoplegia

may further explain our finding that intraoperative platelet transfusions are associated with longer mechanical ventilation and intensive care stay.

However, although propensity score matching resulted in comparable baseline characteristics of both groups, it is also possible that the observed association is due to residual confounding, which is not visible in the measured baseline characteristics. Moreover, most of the observed associations are not strong so they might also be explained by random chance and then it would be incorrect to reject the null hypothesis (type I error). We did not adjust the *P* values in tables 2 and 3 for multiple testing, although we analysed multiple endpoints. If Bonferroni correction had been used, the associations between an early platelet transfusion and amount of blood loss; postoperative mechanical ventilation; intensive care stay; vasoactive drugs; and erythrocyte administration in the ICU would no longer be considered statistically significant. However, the associations between an early platelet transfusion and postoperative plasma and platelet transfusions in the ICU would remain statistically significant after Bonferroni correction. With considering the results of a Bonferroni correction, we reduce the chance on making type I errors, but risk missing subtle associations with potential clinical importance.

In our study, an early platelet transfusion did not seem to reduce the need for reinterventions for bleeding or tamponade, but was associated with a lower blood loss and a higher rate of erythrocyte, plasma, and platelet transfusions in the ICU. The fact that no statistically significant association was observed between an early platelet transfusion and early reexploration for bleeding and/or tamponade and late intervention for tamponade, might be explained by a lack of statistical power. A possible explanation for the higher rate of postoperative transfusions is that once one transfusion has been administered, the threshold for subsequent transfusions is lowered. Theoretically, a difference in preferences and convictions of treating physicians regarding transfusions, resulting in comparable patients receiving different treatment, could explain the higher transfusion rate in the ICU. However, in practice physicians who made the decision to transfuse platelets in the operating room were generally not responsible for the treatment in the ICU. Finally, in addition to all the above-mentioned considerations, it may also be the case that one postoperative endpoint influenced another postoperative endpoint, but this could not be verified in this database. For example, besides the early platelet transfusion, the plasma and platelet transfusions given in the ICU may also have contributed to the lower blood loss in the intervention group.

Comparison with previous studies

Several other studies have analysed the association between platelet transfusions and morbidity and mortality in cardiac surgery with varying findings. Our results are in contrast with the studies that report that transfusion of platelets increases the risk

of serious adverse outcomes.[17,20,21,32] There are various possible explanations for the discrepancy between the findings of these studies and our results. First, not in all studies appropriate and sufficient adjustment of potential confounding factors, like use of aprotinin or concomitant erythrocyte and plasma transfusions, was applied. Second, in contrast to these four studies, we aimed at analysing patients who only received one platelet unit and no other blood product shortly after end of CPB while still in the operating room. We focused on these patients because the indication for the platelet transfusion can be debatable and these patients are most comparable to patients who received no transfusion. Third, a considerable part of the platelet units examined in these studies was not leukocyte-reduced, and the vast majority of the units we studied were leukocyte-reduced.

Our results are consistent with several studies that showed no correlation between platelet transfusion and adverse outcomes like infection, low cardiac output syndrome, MI, stroke, renal failure, sepsis, and mortality.[15,18,33] One study ascertained an association between perioperative platelet transfusion and an increased risk of surgical reexploration for bleeding, which we did not observe. However the remaining results of this study, regarding postoperative mortality, composite endpoint, infectious, cardiac, renal, pulmonary and neurologic complications, were similar to ours.[19]

Strengths and limitations

To the best of our knowledge, this study is the first to analyze the effect of a single platelet transfusion in a broader cardiac surgery population, consisting both of patients who underwent coronary artery bypass graft and those undergoing (concomitant) valve procedures. Besides we report not only on adverse outcomes but also the intended effect of the transfusion, which is to prevent and/or stop

excessive bleeding. Hereby, we aimed at obtaining the overall picture of all potential consequences for a clinician who is considering a platelet transfusion for a cardiac surgery patient. A potential concern of our study is the 16-yr period that was studied because multiple developments occurred both in blood banking and in cardiac surgery and anesthesiology in this period. However, by including year of surgery in the propensity score, we strongly reduced the potential confounding impact of the developments. Furthermore, to the extent of our knowledge, we are the first to study patients who received just a single early platelet transfusion in the absence of concomitant transfusion of other blood products, which precludes potential influence of erythrocyte or FFP transfusions on the outcomes. Another strength of our study is that we adjusted for confounding by indication by propensity score matching. By using propensity score matching, we were able to identify the patients, out of the 17,747 selected patients, who received no early transfusion who were most comparable to the patients transfused

with a single early platelet concentrate. A limitation of our study is that despite accurate propensity score matching, residual confounding, by unknown confounders, cannot be completely ruled out. The large comprehensive cohort of 23,860 patients allowed the analysis of sufficient patients numbers after strict selection of the specific population of interest. Although for some endpoints, the 95% CI was relatively wide, which may be partially caused by a lack of power. Since the database was extensive and detailed, it was possible to include the factors that were considered relevant in the propensity score. Furthermore, since the data had been collected before and therefore independently of the current study, information and selection bias are minimum.

In this study, cardiac surgery patients receiving platelet transfusion in the operating room experienced less blood loss and required vasoactive medication, prolonged ventilation, prolonged intensive care and blood products more often postoperatively. However, our findings further show that an early platelet transfusion was not associated with other serious adverse outcomes like thromboembolic complications, infections, organ failure, in-hospital mortality, and reinterventions for bleeding.

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CHAPTER 6

Storage duration of platelet concentrates and clinical outcomes in cardiac surgery patients

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Background

Storage of platelet concentrates leads to "platelet storage lesion". In transfused patients this may influence haemostatic capacity and adverse events. We set out to investigate in cardiac surgery patients whether longer storage of platelet concentrates is associated with efficacy and safety.

Methods

Using an emulated trial design, we analysed data from patients of two hospitals from January 2005 to December 2017. We included cardiac surgery patients transfused with pooled platelet concentrates. Storage times were classified in 1-3 days (*fresh* platelets) and 4-7 days (*old* platelets). Endpoints were blood loss within 12 hours after surgery, reoperation for bleeding, stroke, myocardial infarction, infection, systemic inflammatory response syndrome, shock, multiorgan failure, and in-hospital mortality. Associations between storage duration and clinical endpoint incidences were quantified using logistic regression corrected for potential confounders.

Findings

In-hospital mortality among 2117 patients transfused with *old* platelets was 10•0% (212 patients); among 1439 patients who received *fresh* platelets it was 7.6% (109 patients); corrected odds ratio (cOR) 1•47, 95% confidence interval (CI) 1•13-1•91. Patients transfused with *old* platelets more often experienced blood loss ≥1000mL (102/285; 35•6%) than patients transfused with *fresh* platelets (87/326; 26•7%), cOR 1•74 (95%CI 1•19-2•52). Patients transfused with *old* platelets more often needed reoperation for bleeding (99/285; 34•7%) than patients transfused with *fresh* platelets (87/326; 26•7%) (cOR 1•62, 95%CI 1•12-2•35). There was no notable association with the other endpoints.

Interpretation

In conclusion, in our cardiac surgery population transfusion of *old* platelets was associated with higher in-hospital mortality, more blood loss, and more reoperations for bleeding compared with *fresh* platelets.

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Rationale

Patients undergoing cardiac surgery are at risk of excessive bleeding and surgical re-exploration. Both excessive blood loss and surgical re-exploration are associated with increased postoperative mortality and morbidity.[1-3] Thus, efficient prevention and treatment of bleeding is an important issue in cardiac surgery. Surgically induced injury is the most important cause of postoperative bleeding. In addition, a significant proportion of the observed bleeding can be explained by acquired haemostatic defects.[4,5] Impaired platelet function, mainly due to the effects of cardiopulmonary bypass and preoperative anti-platelet drug therapy, is one of the most important haemostatic factors leading to postoperative bleeding.[4-8] Platelet transfusions are administered to prevent or treat such bleeding.[9]

A possible adverse effect of *red blood cell* storage duration on clinical outcome in cardiac surgery patients has been studied extensively.[10-13] A possible detrimental effect of storage duration of platelet concentrates on safety and efficacy has been suggested but, thus far, scarcely supported by quantitative clinical evidence.[14]

In vitro studies show that, during storage, platelets undergo multiple changes in structure and function collectively known as "platelet storage lesion".[15-17] Such damage due to storage may reduce haemostatic capacity of the platelet concentrate.[18] Most clinical studies addressing hemostatic capacity this subject have been performed in non-bleeding haemato-oncological patients to treat thrombocytopenia. In a systematic review, transfusion of *old*er platelets has been associated with a shorter time to the next transfusion, a tendency towards a higher risk of bleeding, and, in haemato-oncological patients, an increased need for platelet transfusions.[14] However, whether these findings can be extrapolated to cardiac surgery patients is debatable. Besides leading to reduced haemostatic capacity, several features of the preparation and storage processes of platelet concentrates, lead to platelet activation.[19] Activated platelets might have improved haemostatic effects, but they also show increased CD62P exposure and GPIbα release which is associated with decreased platelet survival after transfusion.[20,21] It is unclear whether platelet activation affects outcomes of cardiac surgery patients and if so whether platelet activation has a favourable or an detrimental effect in these actively bleeding patients. Another concern is the release and accumulation of bioactive substances, like sCD40L and microparticles, during storage of platelets.[18,22-24] These bioactive substances are associated with pro-inflammatory and thrombotic events.[18,25-27]

Our aim was to examine in cardiac surgery patients whether transfusion of platelet concentrates, stored for more than three days, is associated with the occurrence of in-hospital mortality, (excessive) bleeding, reoperation for bleeding, infectious, inflam-

matory, and thromboembolic complications, when compared with administration of platelets with shorter storage duration.

Methods

Study design and patient population

We used an emulated trial design, a method that is suited to simulate a randomised trial when a trial is not feasible, in a cohort of adult patients who underwent cardiac surgery and received one or more platelet transfusions in the Amphia Hospital in Breda, the Netherlands (hospital 1), between January 2005 and December 2017, or in the Leiden University Medical Center (LUMC; hospital 2) in Leiden, the Netherlands, between January 2006 and December 2017. We excluded patients who received apheresis platelets because in the Netherlands apheresis platelets are used for specific indications and tend to be transfused at different storage times and could thereby blur the results. Pseudonymized patient data were obtained from the two hospitals. Decisions regarding patient care were made by the responsible physician according to the applicable guidelines; transfusions were prescribed based on the hemodynamic and haemostatic status of the patient. Decisions regarding platelet transfusion were made independently from the duration of storage of the platelet concentrates, as the physicians are not aware of and cannot influence the storage time of the transfused concentrates. As soon as a platelet concentrate is ordered the concentrate is issued by the blood transfusion department according to the first-in-first-out principle. The medical ethical committees of the Amphia Hospital and of the LUMC approved the study protocol and granted a waiver for informed consent (reference P14.008).

Platelet product

The studied transfused platelet concentrates were all prestorage leukoreduced by filtration, prepared using five whole-blood-derived buffy coats collected and produced by Sanquin Blood Bank (Amsterdam, the Netherlands). The platelets were resuspended either in 100% plasma from one of the five whole blood donations (plasma-platelets) or in 65% platelet additive solution (PAS) and 35% plasma. Two types of PAS were used during the studied period: PAS-B (also known as PAS-2 or T-Sol, Baxter (Nivelles, France)) and PAS-C (also known as PAS-III or Intersol, Fenwal, a Fresenius company, La Châtre, France). The platelets transfused in hospital 1, from January 2005 to December 2012, were stored up to five days in PAS-B and from December 2012 they were stored up to seven days in PAS-C. Platelets transfused in hospital 2, from January 2006 to November 2014, were stored up to seven days in plasma and from November 2014 they were stored up to seven days in platelet additive solution C (PAS-C). Data regarding platelet products were extracted from the national database of Sanquin Blood Bank (eProgesa,

MAKsystems, Paris, France). The storage time of the platelets concentrates was defined as the number of calendar days between the first donation contributing to the pool of five donors and the date of transfusion, such that the donation date is day 0. The storage times were classified in two categories: 1 to 3 days, called *fresh* platelets throughout this manuscript, and more than three days, called *old* platelets. Patients transfused with multiple platelet concentrates were only included if all concentrates were in the same storage time category.

Postoperative clinical outcomes

We planned to evaluate the following endpoints for all patients in both hospitals: amount of blood loss within 12 hours after the end of surgery, reoperation for bleeding, stroke, myocardial infarction, infection, systemic inflammatory response syndrome, shock, multiorgan failure, and in-hospital mortality. In-hospital mortality was available in both hospitals; all other postoperative endpoints were only structurally available in hospital 1. The postoperative endpoints from hospital 1 were retrieved from its prospective peri-operative clinical data registry which is fully compliant with the national cardiac surgery data registry.[28] Herein stroke was defined as a new persistent cerebrovascular event leading to neurologic defects and was diagnosed by a neurologist. Acute kidney failure was defined as the need for postoperative renal replacement therapy when this was not indicated before and/or an increase in serum creatinine of more than 100%. The diagnosis postoperative myocardial infarction was made based on either the occurrence of new Q-waves on the electrocardiogram or ischemic ST-changes in combination with abnormal postoperative troponin-T levels (troponin-T level > 0•5 µg/L for coronary artery bypass grafting (CABG) surgery, troponin-T level > 0•8 µg/L for valve surgery and troponin-T level > 1•0 µg/L for combined CABG and valve procedures). Both serum creatinine and troponin-T were routinely measured in all patients postoperatively. Infection was categorized as pneumonia, mediastinitis, sepsis, and other infections. Diagnosis was stated if relevant organisms were isolated from culture(s). Systemic inflammatory response syndrome was diagnosed if two or more of the following criteria were present: temperature greater than 38 or less than 36°C; tachypnea (greater than 20 breaths per min) or hypocapnea (pCO_2 less than 32 mmHg); tachycardia (greater than 90 beats/min); or need for mechanical ventilation and leukocyte count greater than 12 or less than 4 $\times 10^9$/L. Multiorgan failure was defined as simultaneous or sequential dysfunction or failure of two or more organ systems. Shock was identified as a clinical diagnosis made by the attending physician and as registered in the clinical data registry of the hospital. Amount of blood loss was analysed as being high or low in two endpoints, one with 500 mL and one with 1000mL as the cut-off point.

Statistical analyses

Main analyses

A statistical analysis plan was prepared and agreed upon before starting the analyses. Continuous variables were described as mean (and standard deviation) or median (and interquartile range), as appropriate. Categorical variables were described as a percentage. The number of missing values per variable are presented in the supplemental material (sTable 1). Missing postoperative outcome variables were coded as "no", i.e. we assumed the outcome had not occurred. For the preoperative variable EuroSCORE I there were eight patient records with missing values. These values were imputed using the median of the non-missing. The missing values for the preoperative variables left ventricular (LV) function and recent myocardial infarction were not imputed; in the logistic regression models missingness was handled as a separate indicator category.

The associations between storage time of the platelet concentrate and the clinical endpoint incidences were evaluated using logistic regression derived odds ratios (ORs) with 95% confidence intervals (CIs). We calculated crude and corrected odds ratios, which were corrected for logistic EuroSCORE I (continuous), number of transfused platelet concentrates (continuous), age (continuous), female sex (yes/no), LV function (good (defined as >50%) / not good (<50%) / missing), recent myocardial infarction (yes/no/missing), isolated CABG (yes/no), and center (hospital 1/hospital 2). EuroSCORE I is a scoring system for the prediction of early mortality in patients undergoing cardiac surgery based on objective risk factors.[29]

Kaplan-Meier curves were made in which mortality was compared between patients receiving *fresh* platelets and patients receiving *old* platelets during the first 100 postoperative days. The adjusted Kaplan Meier curves were adjusted using inverse probability weighing. First, we calculated for all patients their predicted estimates of receiving *old* platelets using a logistic regression model with storage time (*old*/*fresh*) as dependent variable and all confounders (listed in the above) as independent variables; next, weights were assigned to all patients as the inverse of those predicted values. The presented adjusted Kaplan Meier curves are thereby corrected for the described confounders.[30]

For the analysis in which we studied mortality, we included all patients who had received platelets at any time during their hospital stay. For all other postoperative endpoints, we restricted the analysis to patients who had received platelet transfusions intraoperatively to assure that the transfusion took place before the occurrence of the endpoint. To evaluate whether the results were similar for different storage media and hospital, we repeated the analyses stratified for storage medium and hospital.

Sensitivity analyses

To explore the robustness of our findings we performed sensitivity analyses. We repeated the analyses in the selection of patients who had received not more than one platelet concentrate during their hospital stay. Also, we repeated the analysis in patients without any missing value for postoperative outcomes (supplemental material).

Role of the funding source

Three authors are employees of Sanquin, the funding source. All authors had full access to the data and the corresponding author had final responsibility for the decision to submit for publication.

Results

Patient characteristics

The database comprised a total of 33,758 (24,769 hospital 1 and 8,989 hospital 2) cardiac surgery patients, of whom 4,317 received one or more platelet transfusions. After exclusion of patients transfused with apheresis platelets and patients transfused with a mix of platelets of different storage time categories, 3,556 patients were included in the analyses (Figure 1). The baseline characteristics per storage time category are shown in Table 1. A small majority of the patients was male (56%), the mean age was 67 years (standard deviation 11 years) and most patients had a good left ventricular function (69%). In total, 2,117 patients were transfused with *old* platelets and 1,439 with *fresh* platelets. Most patients, 2,452/3,556 (69%) patients, received only one platelet concentrate during admission (Figure 1).

Platelet storage duration and in-hospital mortality

Among patients transfused with *old* platelets 212/2,117 (10•0%) died in the hospital whereas among those who received *fresh* platelets 109/1,439 (7•6%) died in the hospital; corrected odds ratio (OR) 1•47, 95%CI 1•13 to 1•91) (Table 2). Similar results were observed after stratification for storage medium and hospital (Table 2). Figure 2 presents Kaplan-Meier curves for in-hospital mortality after receiving *fresh* and *old* platelets. It shows that the increased mortality among patients transfused with *old* platelets compared to patients transfused with *fresh* platelets occurred largely during the first 20 postoperative days.

Platelet storage duration and post-operative bleeding & other clinical outcomes

Information on both postoperative outcomes and timing of transfusions (given in or outside the operating room) was available for 611 patients in hospital 1 (Figure 1). Patients

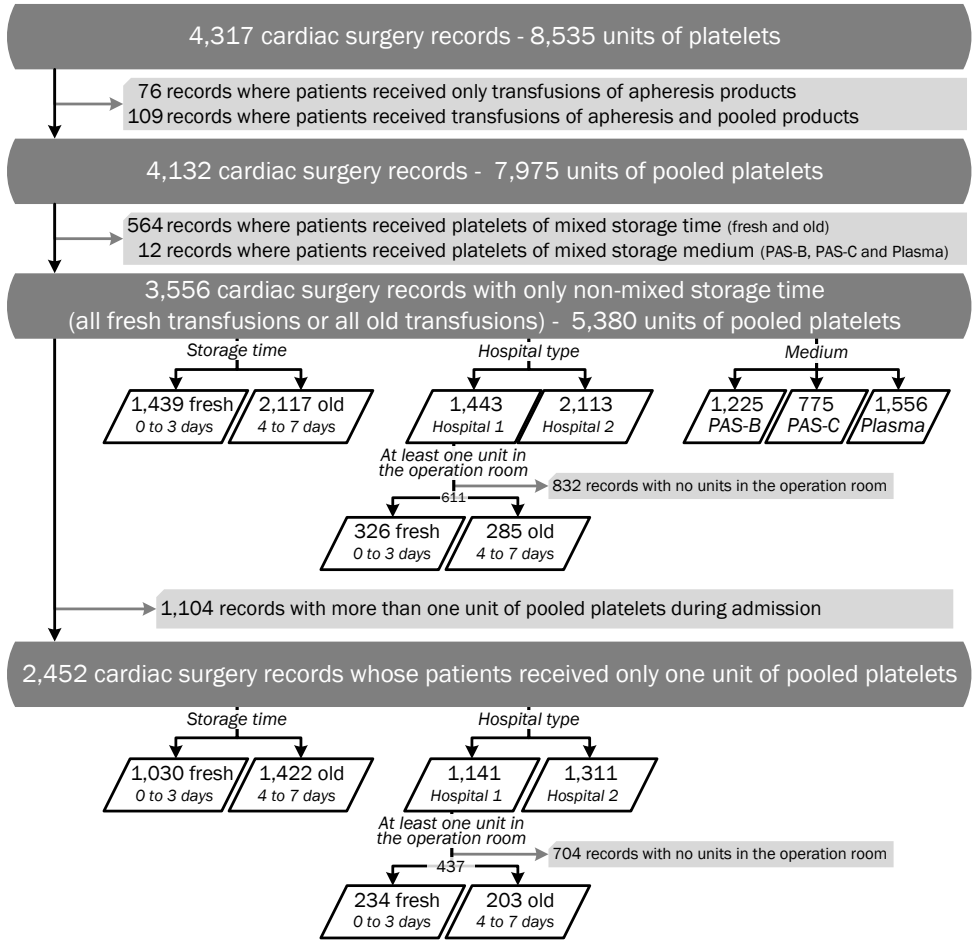

Figure 1: Flowchart of inclusion/exclusion steps

transfused with *old* platelets more often experienced blood loss ≥1000mL (102/285; 35•8%) than patients transfused with *fresh* platelets (87/326; 26•7%), corrected OR 1•74 (95%CI 1•19 to 2•52). Patients transfused with *old* platelets more often needed reoperation for bleeding 99/285 (34•7%) than patients transfused with *fresh* platelets (87/326; 26•7%) (corrected OR 1•62, 95% CI 1•12 to 2•35). There was no clear association between transfusion of *old* versus *fresh* platelets and the occurrence of stroke, myocardial infarction, infection, systemic inflammatory response syndrome, shock, or multiorgan failure (Table 3).

Table 1 - Characteristics and outcomes of cardiac surgery patients receiving platelets

	Storage time		
Characterisitics	**Fresh n=1,439**	**Old n=2,117**	**Overall n=3,556**
Distinct patients	1,434	2,103	3,520
Total – records	1,439	2,117	3,556
hospital 1	777	666	1,443
hospital 2	662	1,451	2,113
One concentrate – total	1,030	1,422	2,452
One concentrate –hospital 1	616	525	1,141
One concentrate – hospital 2	414	897	1,311
Age (years), mean (SD)	67•5 (10•7)	66•3 (11•6)	66•8 (11•2)
Female sex	582 (40•4)	968 (45•7)	1,550 (43•6)
BMI, mean (SD)	26•3 (4•0)	26•5 (4•2)	26•4 (4•1)
Previous cardiac surgery	199 (13•8)	327 (15•5)	526 (14•8)
Creatinine > 200 µmol/L	40 (3•0)	77 (4•0)	117 (3•5)
Active endocarditis	65 (4•7)	82 (4•1)	147 (4•4)
LV function good*	952 (67•0)	1,461 (70•1)	2,413 (68•8)
Recent myocardial infarction*	243 (19•2)	299 (15•8)	542 (17•1)
Emergency surgery	240 (17•4)	362 (18•2)	602 (17•9)
Isolated CABG	611 (42•7)	778 (36•8)	1,389 (39•1)
EuroSCORE I†, median (IQR)	7•9 (3•4 - 18•0)	7•5 (3•3 - 16•5)	7•7 (3•3 - 16•9)
Number of platelet transfusions, median (IQR)	1 (1 - 2)	1 (1 - 2)	1 (1 - 2)
in the operation room, median (IQR) ‡	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)
outside the operation room, median (IQR) ‡	1 (0 - 1)	1 (0 - 1)	1 (0 - 1)
Number of RBC transfusions, median (IQR)	3 (1 - 6)	3 (1 - 6)	3 (1 - 6)
Outcomes	**n=1,439**	**n=2,117**	**n=3,556**
Death	109 (7•6)	212 (10•0)	321 (9•0)
	n=326	**n=285**	**n=611**
Blood loss ≥ 500 mL in first 12 hours‡	173 (53•1)	170 (59•7)	343 (56•1)
Blood loss ≥ 1000 mL in first 12 hours‡	87 (26•7)	102 (35•6)	189 (30•1)
Reoperation for bleeding‡	87 (26•7)	99 (34•7)	186 (30•4)
Stroke‡	10 (3•1))	8 (2•8)	18 (2•9)
Myocardial infarction‡	53 (16•3)	36 (12•6)	89 (14•6)
Infection‡	86 (26•4)	76 (26•7)	162 (26•5)
SIRS‡	67 (20•6)	75 (26•3)	142 (23•2)
Shock‡	85 (26•1)	84 (29•5)	169 (27•7)
Multi organ failure‡	50 (15•3)	36 (12•6)	86 (14•1)

Numbers represent number and percentages unless when stated otherwise

Percentages and statistics are calculated based on non-missing values (total valid observations)

CABG coronary artery bypass graft; IQR interquartile range; LV left ventricle; L liter; SD standard deviation; SIRS systemic inflammatory response syndrome

* Missings are included in the logistic regression models as an indicator category

† Single imputation (median)

‡ Only collected in one hospital and restricted to patients who received platelet transfusions in the operation room, denominators: *fresh*=326, *old*=285 and overall=611

Note: for all but "Distinct patients" all numbers refer to records. Patients are counted twice when there was more than one surgery during the study time.

Sensitivity analyses

Among 2,452 patients who received one concentrate of platelets the association for in-hospital mortality was similar, with a broader, in part non-statistically significant, confidence interval, OR 1•24 (95%CI 0•88 to 1•74), (Table 2). Similar results were observed for blood loss ≥1000mL, OR 1•50 (95%CI 0•94 to 2•37) and for reoperation for bleeding, OR 1•95 (95%CI 1•23 to 3•10), (Table 3). For the other postoperative outcomes (stroke, myocardial infarction, infection, systemic inflammatory response syndrome, shock, or multi organ failure) the analyses among patients transfused with only one platelet concentrate also yielded similar results as the main analysis.

In the main analysis missing postoperative outcome variables were coded as "no", i.e. we assumed the outcome had not occurred. To explore the effect of this assumption we selected the patients who did not have missing values on bleeding complications and repeated the main analysis. The results of this sensitivity analysis among the patients who did have information on blood loss in the first 12 hours ≥500mL, blood loss in the first 12 hours ≥1000mL and/or for reoperation for bleeding were similar to the main analysis, confirming the robustness of our findings (supplemental Table 2).

Table 2 - Odds ratio for in-hospital mortality; *old* vs *fresh* concentrates in the total population and stratified per storage medium and hospital

	Number of deaths / number of patients (% deaths)		OR (95% CI) of *old* vs *fresh* platelets	
	Fresh	**Old**	**Crude**	**Corrected***
Overall	109/1,439 (7•6)	212/2,117 (10•0)	1•36 (1•01 to 1•73)	1•47 (1•13 to 1•91)
Storage fluid				
PAS-B	64/711 (9•0)	65/514 (12•7)	1•46 (1•02 to 2•11)	1•49 (1•00 to 2•21)
PAS-C	19/230 (8•3)	59/545 (10•8)	1•35 (0•78 to 2•32)	1•36 (0•73 to 2•52)
Plasma	26/498 (5•2)	88/1,058 (8•3)	1•65 (1•05 to 2•59)	1•88 (1•16 to 3•03)
Hospital				
Hospital 1	73/777 (9•4)	85/666 (12•8)	1•41 (1•01 to 1•97)	1•41 (0•99 to 2•02)
Hospital 2	36/662 (5•4)	127/1,451 (8•8)	1•67 (1•14 to 2•44)	1•72 (1•15 to 2•59)
Only one concentrate				
Overall	67/1,030 (6•5)	104/1,422 (7•3)	1•13 (0•83 to 1•56)	1•24 (0•88 to 1•74)
Hospital 1	48/616 (7•8)	53/525 (10•1)	1•33 (0•88 to 2•00)	1•26 (0•82 to 1•95)
Hospital 2	19/414 (4•6)	51/897 (5•7)	1•25 (0•73 to 2•15)	1•31 (0•74 to 2•31)

CI confidence interval; OR odds ratio; PAS platelet additive solution

*corrected for number of transfusions, logistic EuroSCORE I, age, gender, left ventricular function, recent myocardial infarction isolated CABG, and hospital (unless the OR was already stratified for hospital)

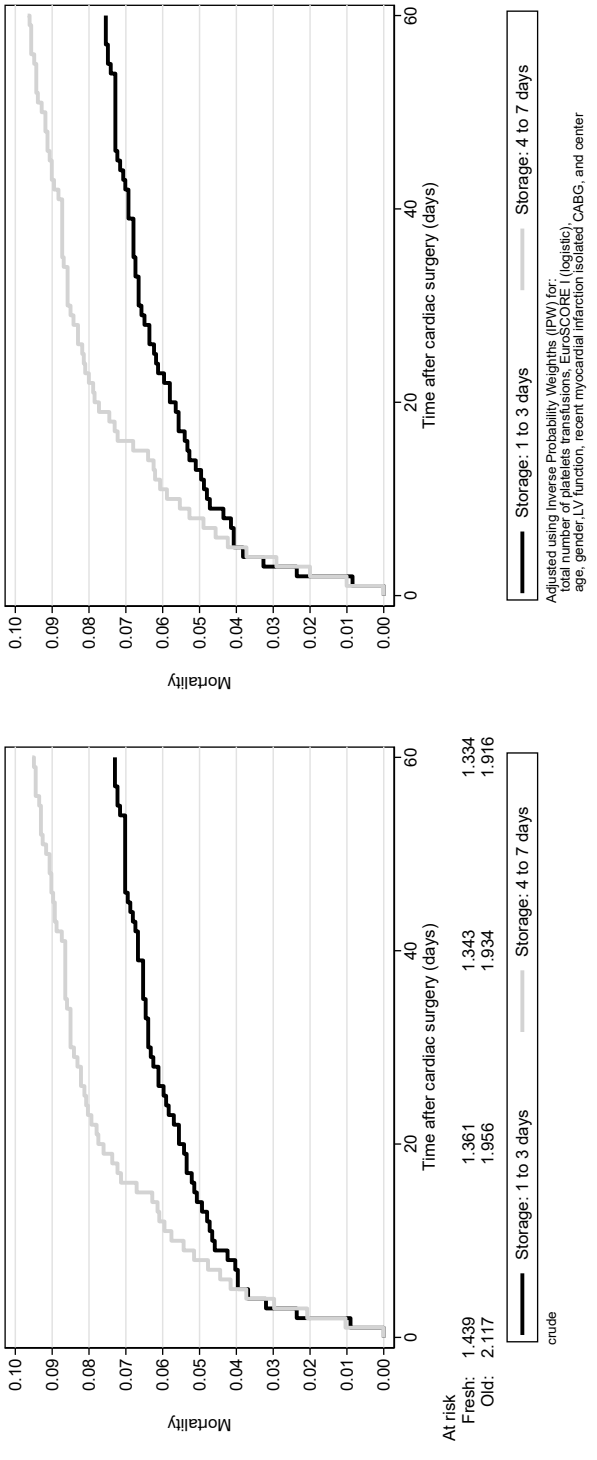

Figure 2: Crude and adjusted Kaplan-Meier curves for the occurrence of in-hospital mortality according to time (days) after cardiac surgery.

Table 3 - Odds ratio for postoperative outcomes according to administrating *old* vs *fresh* concentrates

	Number of outcomes / number of patients (% outcome)		OR (95% CI) of *old* vs *fresh* platelets	
	Fresh	Old	Crude	Corrected*
Other outcomes				
Blood loss ≥ 500 mL first 12 hours†	173/326 (53•0)	170/285 (60•0)	1•31 (0•95 to 1•80)	1•38 (0•98 to 1•92)
Blood loss ≥1000 mL first 12 hours†	87/326 (26•7)	102/285 (35•8)	1•53 (1•08 to 2•16)	1•74 (1•19 to 2•52)
Reoperation for bleeding†	87/326 (26•7)	99/285 (34•7)	1•46 (1•03 to 2•07)	1•62 (1•12 to 2•35)
Stroke†	10/326 (3•1)	8/285 (2•8)	0•91 (0•35 to 2•34)	0•90 (0•34 to 2•33)
Myocardial infarction†	53/326 (16•3)	36/285 (12•6)	0•74 (0•47 to 1•18)	0•87 (0•62 to 1•21)
Infection†	86/326 (26•4)	76/285 (26•7)	1•01 (0•71 to 1•46)	0•99 (0•68 to 1•44)
SIRS†	67/326 (20•6)	75/285 (26•3)	1•38 (0•95 to 2•01)	1•35 (0•92 to 1•99)
Shock	85/326 (26•1)	84/285 (29•5)	1•18 (0•83 to 1•69)	1•16 (0•79 to 1•69)
Multi organ failure	50/326 (15•3)	36/285 (12•6)	0•80 (0•50 to 1•27)	0•73 (0•45 to 1•19)
Other outcomes - Only one concentrate				
Blood loss ≥ 500 mL first 12 hours‡	108/234 (46•2)	107/203 (52•7)	1•30 (0•89 to 1•90)	1•35 (0•92 to 1•98)
Blood loss ≥1000 mL first 12 hours‡	47/234 (20•1)	54/203 (26•6)	1•44 (0•92 to 2•25)	1•50 (0•94 to 2•37)
Reoperation for bleeding‡	44/234 (18•8)	58/203 (28•6)	1•73 (1•10 to 2•70)	1•95 (1•23 to 3•10)
Stroke‡	5/234 (2•1)	7/203 (3•5)	1•64 (0•51 to 5•24)	1•78 (0•55 to 5•81)
Myocardial infarction‡	37/234 (15•8)	24/203 (11•8)	0•71 (0•41 to 1•24)	0•63 (0•35 to 1•15)
Infection‡	57/234 (24•7)	48/203 (23•7)	0•96 (0•62 to1•49)	0•88 (0•56 to 1•38)
SIRS‡	49/234 (20•9)	56/203 (27•6)	1•44 (0•93 to 2•23)	1•50 (0•95 to 2•36)
Shock‡	57/234 (24•4)	54/203 (26•6)	1•13 (0•73 to 1•73)	1•09 (0•69 to 1•74)
Multi organ failure‡	32/234 (13•7)	22/203 (10•8)	0•77 (0•43 to 1•37)	0•71 (0•39 to 1•31)

CI confidence interval; OR odds ratio; SIRS systemic inflammatory response syndrome

*corrected for number of transfusions, logistic EuroSCORE I, age, gender, left ventricular function, recent myocardial infarction, and isolated CABG

† restricted to patients who received one or more platelet transfusions in the operation room

‡ restricted to patients who received one platelet transfusion in the operation room

Discussion

The main finding of this study is that, in a cardiac surgery population, transfusion of *old* platelets was associated with a higher in-hospital mortality when compared to transfusion of *fresh* platelets. Moreover, patients transfused with *old* platelets more often developed blood loss ≥1000 mL in the first 12 hours after surgery and more often required reoperation for bleeding than patients transfused with *fresh* platelets. In our study hospital mortality was higher than in the general cardiac surgery population,

which is explained by the specific population that was selected. Patients who have an indication for platelet transfusion have a worse clinical condition than patents who don't require platelet transfusions.

We selected cardiac surgery patients who received solely *old* platelets or solely *fresh* platelets. That enabled a clear comparison without any influence of exposure to both types of platelets. We explored possible differences between platelet storage fluids and per hospital. Similar results in those subgroups confirmed that storage duration was indeed associated with mortality and bleeding outcomes in various settings. Differences in outcomes between patients exposed to platelets with different storage time may have been due to other risk factors for the outcome. Yet, clinical decisions to transfuse *fresh* or *old* platelet concentrates were made independently from the storage time of the platelet concentrates, as the treating physicians was not aware of and could not influence the storage time of the transfused concentrates. Consequently, one can assume that assignment to *fresh* or *old* platelets was independent of the clinical condition of the patient. This is confirmed by the results in Table 1, showing that risk factors for mortality and clinical outcomes did not differ between patients treated with *fresh* and *old* platelet concentrates. Platelets are issued according to the first-in-first-out principle. Therefore, the storage time can be influenced by the number of platelets a patient receives, because the more concentrates a patient received, the bigger the chance that that patient will receive a *fresh* concentrate (as these concentrates lie in the back of the shelf). Therefore, patients with unfavourable risk profiles and worse prognosis tend to receive more platelet concentrate units with a higher chance of receiving *fresh*er platelets. To minimize this potentially confounding factor of the amount of platelet transfusions, our analyses were corrected for number of platelet transfusions administered. Even if residual confounding remained despite the applied correction, then we expect it to result in an underestimation of the actual effect, because the patients with a less favourable prognosis were more likely to receive younger platelets than the patients with a better prognosis. The analyses were also corrected for "logistic EuroSCORE I", age, gender, left ventricular function, recent myocardial infarction, isolated CABG, and hospital. EuroSCORE I is an internationally accepted scoring system for the prediction of early mortality in cardiac surgical patients (in Europe) based on objective preoperative risk factors.[29] So by correcting for EuroSCORE I factors that are considered relevant for early mortality in cardiac surgery are taken into account. We therefore conclude that it is unlikely that bias or confounding explain our findings.

The literature on the association between platelet storage time and clinical outcomes after thoracic surgery is scarce. A systematic review and meta-analysis that evaluated the effect of platelet storage time on platelet measurements (count increment, corrected count increments, platelet recovery, survival and half-life) after platelet transfusion[31]

concluded that *fresh* platelets were superior to *old* platelets for all these laboratory platelet measurements. Another systematic review and meta-analysis examined the effect of storage time of transfused platelet concentrates on the clinical outcomes.[14] This review concluded that prolonged storage decreases the efficacy of platelet transfusions, resulting in a shorter interval to the next transfusion. Also, a trend towards a higher risk of bleeding and an increased need of platelet transfusions was observed. However, most of the papers included in this review describe the outcomes of haematological patients. Results in this patient population may not be transportable to cardiac surgery patients. Another systematic review also studied the association between storage time of platelets and clinical endpoints.[32] In this review most studies, 13 of 18, concerned haematological patients, and five of the 18 studies described critically ill patients. Critically ill patients consisted of trauma patients, cardiac surgery patients and a heterogeneous population of critically ill patients. The conclusions were that no association was found between storage time of platelets and clinical outcomes, including bleeding, sepsis, or mortality. This paper also stated that there is an absence of evidence to draw definitive conclusions, especially in critically ill patients. So, both reviews don't yield clear clinical results that apply to (cardiac) surgery patients.

To the best of our knowledge there is one other study that investigated the association between storage time of platelets and clinical outcomes of cardiac surgery patients.[33] In contrast to our study, this study did not find a statistically significant association between storage time of platelets and adverse outcomes. A possible explanation for the difference between the findings is that the study of Welsby et al only contained nonemergent CABG patients transfused with apheresis platelets with a maximum storage of five days while our study included both elective and non-elective cardiac surgery patients transfused with pooled buffy-coat platelets with a maximum storage of seven days. So, our database contains more high-risk patients who might also be more vulnerable for the potential influence of transfusion with *old* platelets. This difference in risk-profile of the patient populations is also reflected in the hospital mortality (of 5•2%) observed by Welsby et al, that is lower than the hospital mortality we observed (9•0%). Besides, possibly the "platelet storage lesion" is different in apheresis than in pooled platelets and the two days longer maximal storage duration in our pooled platelets might lead to more "storage lesion". Our study had a higher statistical power because our main analysis is performed in a larger population and our population consisted of patients transfused solely with *old* or solely with *fresh* platelets to avoid bias.

Our findings suggest that in-hospital mortality, postoperative (first 12 hours) blood loss >1000 mL and reoperation for bleeding occur more frequently following transfusion of *old* platelets. Based on these results the most obvious potential explanation for the higher hospital mortality are higher blood loss and the resulting higher need

for reoperation. If this is the actual explanation for the higher hospital mortality after transfusion with *old* platelets, this would suggest that *old* platelets are less effective in preventing and/or stopping bleeding in cardiac surgery. This possible explanation for our findings is plausible in the light of the discoveries earlier studies uncovered over storage time: increasing platelet activation, decreasing platelet responsiveness to agonist and declining platelet viability and function, together called the "platelet storage lesion".[14,15,17,31,34-36] Besides the possibly reduced efficacy of *old* platelets compared to *fresh* platelets, other possible (partial) explanations of the higher in-hospital mortality could be a higher risk of TRALI, other transfusion reactions, or other complications that were observed with longer storage of platelets.[37-40]

If prolonged storage time indeed negatively affects mortality one might consider to preferably transfuse *fresh*er platelet concentrates to cardiac surgery patients. Yet, a single observation from a non-experimental study does not provide sufficient proof for a change in clinical practice. Therefore, our findings need confirmatory evidence.

In conclusion, in our cardiac surgery population transfusion of *old* platelets was associated with a higher hospital mortality, more blood loss, and more reoperations for bleeding compared with *fresh* platelets. In our opinion these results instigate further research into this topic because if *old* platelets do cause higher hospital mortality in cardiac surgery patients this asks for a change in clinical practice.

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Supplemental material

Table 1 Missings

	Storage time				Overall	
	Fresh		Old			
Preoperative	**n=1,439**	**%**	**n=2,117**	**%**	**n=3,556**	**%**
Age	0	0%	0	0%	0	0%
Gender	0	0%	0	0%	0	0%
BMI	475	33%	1,004	47%	1,479	42%
Previous cardiac surgery	0	0%	0	0%	0	0%
Creatinine > 200 µmol/L	83	6%	171	8%	254	7%
Active endocarditis	60	4%	128	6%	188	5%
LV function good*	17	1%	33	2%	50	1%
Recent myocardial infarction*	171	12%	218	10%	389	11%
Emergency surgery	58	4%	125	6%	183	5%
Isolated CABG	0	0%	0	0%	0	0%
EuroSCORE I†	1	0%	7	0%	8	0%
Number of platelet transfusions	0	0%	0	0%	0	0%
in the operation room‡	0	0%	0	0%	0	0%
outside the operation room‡	0	0%	0	0%	0	0%
Number of RBC transfusions	0	0%	0	0%	0	0%
Postoperative§						
Death	0	0%	0	0%	0	0%
Blood loss ≥ 500 mL in first 12h‡§	14	4%	19	7%	33	5%
Blood loss ≥1000 mL in first 12h‡§	14	4%	19	7%	33	5%
Reoperation for bleeding ‡§	112	34%	86	30%	198	32%
Stroke‡	417	54%	313	47%	730	51%
Myocardial infarction‡	25	3%	82	12%	107	7%
Infection‡	7	1%	9	1%	16	1%
SIRS‡	5	1%	3	0%	8	1%
Shock‡	5	1%	12	2%	17	1%
Multi organ failure‡	4	1%	3	0%	7	0%

Reported percentages refer to the population included in the analyses (see items ‡ and § below)

* Missings are included in the logistic regression models as an indicator category

† Single imputation (median)

‡ Only collected in the hospital 1, denominators: fresh=777, old=666 and overall=1,443

§ Restricted to patients who received one or more platelets transfusions in the operation room, denominators: fresh=326, old=285 and overall=611

Table 2 Sensitivity analyses – Excluding patients who had missings for bleeding-related outcomes

Missing	Storage time				Overall	
	Fresh		Old			
	n=1,439	%	n=2,117	%	n=3,556	%
Outcomes[§]						
Blood loss ≥ 500 mL in first 12h[‡§]	14	4%	19	7%	33	5%
Blood loss ≥1000 mL in first 12h[‡§]	14	4%	19	7%	33	5%
Reoperation for bleeding [‡§]	112	34%	86	30%	198	32%

	Number of outcomes / number of patients (% outcome)		OR (95% CI) of old vs fresh platelets	
	Fresh	Old	Crude	Corrected*
Other outcomes				
Blood loss ≥ 500 mL in first 12h[†]	173/326 (53.0)	170/285 (60.0)	1.31 (0.95 to 1.80)	1.38 (0.98 to 1.92)
Sensitivity analyses[1]	173/312 (55.5)	170/266 (63.9)	1.42 (1.01 to 1.98)	1.51 (1.07 to 2.16)
Blood loss ≥1000 mL in first 12h[†]	87/326 (26.7)	102/285 (35.8)	1.53 (1.08 to 2.16)	1.74 (1.19 to 2.52)
Sensitivity analyses[1]	87/312 (27.9)	102/266 (38.4)	1.61 (1.14 to 2.28)	1.83 (1.25 to 2.69)
Reoperation for bleeding[†]	87/326 (26.7)	99/285 (34.7)	1.46 (1.03 to 2.07)	1.62 (1.12 to 2.35)
Sensitivity analyses[2]	87/214 (40.7)	99/199 (49.8)	1.44 (0.98 to 2.13)	1.70 (1.11 to 2.61)

*corrected by number of transfusions, logistic EuroSCORE, age, gender, LV function, recent myocardial infarction, and isolated CABG

† restricted to patients who received one or more platelet transfusions in the operation room

1 sensitivity analyses: excludes patients with missing data on blood loss. In the main paper they where recoded as "non experiencing the outcome"

2 sensitivity analyses: excludes patients with missing data on reoperation for bleeding. In the main paper they where recoded as "non experiencing the outcome"

CHAPTER 7

General discussion

Aim of this thesis

The overall goal of this thesis was to expand knowledge about the safety and efficacy of platelet transfusions in general and in particular in cardiac surgery patients by studying the influence of the administration of one unit of platelets, the influence of storage medium and storage time on clinical outcomes of patients. Expansion of knowledge about this topic creates possibilities to further improve the safety and efficacy of platelet transfusions.

The first issue this thesis addresses is whether certain characteristics of a platelet unit influence its efficacy and safety. In hematology patients, it has been suggested that reducing the amount of plasma in platelet units could diminish transfusion reactions. However, the results of these studies may not be applicable to other patients receiving platelet transfusions, like trauma or cardiac surgery patients. In this thesis we compared the occurrence of transfusions reactions in all patients transfused with PAS-B-platelets, PAS-C-platelets, or plasma-platelets in routine clinical use.

Besides a possible difference between PAS and plasma-platelets regarding safety, and in particular transfusion reactions, also efficacy is of major concern. It is unclear whether PAS-platelet concentrates are as effective as plasma-platelet concentrates. In our *in-vitro* study we compared the hemostatic function of PAS-platelets to plasma-platelets in reconstituted whole blood. The hemostatic function was measured using Multiplate-derived platelet aggregation and TEG-measured overall clot formation.

Apart from storage medium also the storage time of platelet concentrates is of interest with regard to safety and efficacy of platelet transfusions. Increased storage time has been associated with the accumulation of biological response modifiers, such as inflammatory cytokines and chemokines.(1-4). Whether these changes also have clinical consequences is not clear yet, as published results on this matter are contradictory.(5, 6) This controversy indicates that a better understanding of the influence of storage time on safety and efficacy is needed and will create an opportunity to further improve the effect of platelet transfusions. Therefore, we assessed whether there is an association between the storage time of platelet units and transfusion reactions.

In addition to the characteristics of the platelet concentrate, it is plausible that also the patient characteristics and the underlying clinical situation necessitating platelet transfusion may influence its effect. Since a significant proportion of all platelet transfusions is consumed by cardiac surgery patients, it is important to understand the effect of a platelet concentrate and its storage conditions on cardiac surgery patients. There is a lack of clinical evidence establishing the hemostatic effectiveness of platelet transfu-

sions in cardiac surgery patients.(7) In addition, conflicting results have been reported regarding the safety of platelet transfusions in the setting of cardiac surgery.(8-14) Consequently, there is an unmet need to determine the clinical impact (safety and efficacy) of perioperative platelet transfusions in patients undergoing cardiac surgery. Therefore, we studied the efficacy and safety of platelet transfusion following cardiac surgery by comparing patients who have received a platelet transfusion with propensity-score-matched patients who did not receive a transfusion.

Subsequently, the question arose whether storage time of platelet concentrates has a clinically significant impact in transfused cardiac surgery patients. As mentioned above, *in vitro* studies show that during storage platelets undergo multiple changes in structure and function collectively known as the "platelet storage lesion".(15, 16) It is conceivable that in patients this "platelet storage lesion" results in a reduced hemostatic capacity and more adverse events.(17-19) Hence, we evaluated whether platelet storage time is associated with efficacy and safety outcomes in cardiac surgery patients receiving platelet transfusions.

Discussion and considerations for future research

Part I: Platelet transfusions in general

Impact of storage medium on safety and efficacy

One of the most important findings in our study, in chapter 2, is that PAS-C-platelets are associated with less transfusion reactions than plasma and PAS-B platelets. This result is based on a nationwide database comprising all reported transfusions reactions of a period of 10 years, representing the whole patient population transfused with platelet concentrates in this time frame. The geographical determined distribution of storage media within the Netherlands provided a unique opportunity to compare the storage media within one country. In contrast to most previous studies, the advantages of this setting are that the different storage media are used in comparable populations, during the same time period, within the same healthcare system with the same protocols and the same hemovigilance system. The database covers over half a million platelet transfusions, enabling the analysis of less common reaction types like anaphylactic reactions, TRALI, and TACO separately. Moreover, the overall incidence of reported reactions in our study is relatively high compared to other countries with passive surveillance. The incidence of RBC transfusion reactions is also relatively high which is probably indicative of the accuracy of the Dutch hemovigilance system. The considerable size of our study population made it possible to estimate the effect of storage medium on the incidence of transfusion reactions with more certainty and accuracy than previous studies.

In our *in vitro* study, in chapter 3, we observed that the aggregation and the agonist induced CD62P responsiveness of PAS-C-platelets was lower than the aggregation of plasma–platelets. However, TEG-derived clot formation, showed no differences between the PAS-C-platelets and plasma-platelets. This might suggest that the decreased platelet aggregation does not result in impaired overall clot formation. However, another possibility is that TEG is (partly) insensitive to differences in platelet function. In viscoelastic testing the applied activators give rise to a thrombin burst that can bypass and thereby hide dysfunction of adenosine diphosphate receptors by activating protease-activated receptors (PAR).(20)

Besides the lower platelet function of PAS-C-platelets compared to plasma-platelets, also the fluid in which the platelets are stored may be an issue of concern. While plasma-platelets are stored in donor plasma containing coagulation factors, in PAS-platelets most of the plasma is replaced by PAS-fluid, which does not contain coagulation factors. In case of massive transfusion the lower concentration of coagulation factors in PAS-C could pose a problem, as was also discussed in the 2015 review of Dudaryk et al.(21) We propose that future research should compare the clinical (bleeding related) outcomes of massive transfusions with PAS-platelets to massive transfusions with plasma-platelets. An advantage of performing such a study retrospectively with Dutch data is that PAS and plasma stored platelets were used concurrently in the Netherlands.

Impact of storage time on safety and efficacy of platelets

Our study, in chapter 4, exploring the effect of storage time on transfusion reaction rates was performed on a large nationwide database, comparable to the database used for the storage medium (see previous paragraph).

We demonstrated that in contrast to plasma stored platelets, in PAS platelets, storage time is associated with a higher incidence of transfusion reactions. However, the overall incidence of transfusion reactions, so regardless of storage time, following plasma platelets is higher than that of PAS-C platelets.

Besides the safety aspect of platelet transfusions, obviously the intended hemostatic efficacy should also be considered. Both PAS-platelet and plasma-platelets showed an increase in baseline CD62P expression, indicating baseline activation, with increasing storage time. Also, a decrease in CD62P responsiveness over storage time was observed. In another study a correlation was observed between platelet responsiveness (TRAP induced) and 1-hour corrected count increment (CCI).(22) The CCI is the increase in circulating platelets corrected for the administered dose and the blood volume of the patient and is often used for hemato-oncological patients. Despite being a poor surrogate marker for bleeding tendency,(23-25) CCIs are associated with the time to next

transfusions and are therefore of clinical relevance (26). Whether the decreasing CD62P responsiveness over storage time is also associated with hemostatic efficacy parameters in (cardiac) surgery patients is unknown.

Furthermore, both in plasma-platelets and in PAS-platelets (Multiplate-derived) *in vitro* aggregation (TEG-derived) initial clot formation significantly declined with an increasing storage time, but (TEG-derived) maximum clot strength and clot growth rate did not decline with increasing storage time. Furthermore, In previous studies, Multiplate results have been shown to be correlated to bleeding and thromboembolic complications in patients undergoing coronary stenting and cardiac surgery.(27-29)

Evidently, further research is needed to explore whether the changes we observed *in vitro* also cause changes in clinical practice, where other factors like blood flow, vascular endothelium, other blood components, and patient factors influence hemostasis and outcomes. The potential effect of storage time of could be different in different patient populations, so these clinical studies should be performed in separate populations receiving platelet transfusions.

Part II: Platelet transfusions in cardiac surgery patients

Impact of a platelet transfusion on safety and efficacy

In addition to the characteristics of the platelet concentrate, it is plausible that also the patient characteristics and the clinical situation influence the effect of a platelet transfusion. To the best of our knowledge, our study in chapter 5 is the first paper describing not only the adverse outcomes, but also the intended effects of a platelet transfusion in cardiac surgery patients and thereby presenting an overall picture of the outcomes following platelet transfusion. In this retrospective analysis, cardiac surgery patients receiving platelet transfusion in the operating room experienced less blood loss, but more often required vasoactive medication, prolonged ventilation, prolonged intensive care admission, and transfusion of other blood products postoperatively. However, early platelet transfusion was not associated with reinterventions, thromboembolic complications, infections, multi-organ failure, or mortality.

In contrast to other studies we analyzed patients receiving only one platelet unit and no other blood product shortly after the end of cardiopulmonary bypass(CPB). We selected these patients because in these patients the indication for the platelet transfusion varies between physicians. As a result, patients receiving one unit and those receiving no units have more or less similar clinical characteristics, which reduces the chance of confounding-by-indication. In addition, we used propensity score matching to identify

the patients, who received no transfusion who were most comparable to the patients transfused with a platelet concentrate and thereby reduce the potential remaining confounding even further. Furthermore, our data were collected prior to and therefore independently of our analysis, minimizing information and selection bias. However, larger studies are necessary to estimate the impact of platelet transfusions in cardiac surgery patients. Ideally, future research should prospectively compare a restrictive with a liberal platelet transfusion policy.

Indication for a platelet transfusion in cardiac surgery

Besides the impact of a platelet transfusion in cardiac surgery, the exact indication for platelet transfusion in cardiac surgery patients should also be established. Transfusing the right patients at the right time, is an essential and challenging part of transfusion medicine. In cardiac surgery there is a lack of guidance on platelet transfusion. To our knowledge, there are no randomized controlled trials about the safety and efficacy of platelet transfusions in cardiac surgery.(30) The scarcity of evidence to guide the use of platelets in cardiac surgery contributes to the significant variability in transfusion practice in cardiac surgery patients.(31-33). The American Association of Blood Banks (AABB), EACTS/EACTA and STS/ACS guidelines provide recommendations based on low-quality evidence and the existing guidelines provide little concrete insights with respect to the treatment of postoperative bleeding when (likely) related to platelet dysfunction. The EACTS/EACTA guidelines propose that platelet concentrate should be transfused in bleeding patients with a platelet count below 50, 000 cells/μL or under antiplatelet therapy (class IIa, level C).(34) According to the STS/ACS guidelines the decision to transfuse non-red cell hemostatic blood products should be based on clinical evidence of bleeding and preferably guided by point-of-care tests that assess hemostatic function in a timely and accurate way. (class IIA, level of evidence C).(30) However, neither specific tests nor specific values for platelet dysfunction are advised. Accordingly, as was observed in a study evaluating the changes in clinical practice as a result of the guidelines, the influence of the (STS/ACS) guidelines on daily patient care is limited. (35) The authors suggested several possible explanations, including "practitioner views of outside control of clinical practice, low level of strength of the evidence supporting a given recommendation, appropriateness and usefulness of the specific guidelines, and availability of resources to implement the guidelines". So, currently the indication for platelet transfusions is still significantly influenced by the institution, the physician and other subjective, immeasurable factors such as the surgeon estimation of excess bleeding and (previous) antiplatelet medication use by the patient without an actual measurement of the platelet function.(31) The resulting under- and overutilization of platelet transfusions in cardiac surgery should be avoided because underutilization can lead to unnecessary blood loss and associated morbidity and overutilization will lead to unnecessary adverse events and costs.

POC tests and transfusion algorithms in cardiac surgery

In the STS/ACS guidelines "a multimodality approach involving multiple stakeholders, institutional support, enforceable transfusion algorithms supplemented with point-of-care testing, and all of the efficacious blood conservation interventions" as this 'will limit blood transfusion and provide optimal blood conservation for cardiac operations" (class I recommendation, level of evidence A). The rationale for this recommendation is based on a number of studies prospectively analyzing transfusion algorithms in institution-derived transfusion practices in concurrence with accurate point-of-care (POC) testing to handle bleeding and to guide blood transfusion. Most of the mentioned studies (36-41), including two RCTs, showed that the use of POC testing and transfusion algorithms resulted in improved hemostasis and in fewer transfusions. Also the EACTS/EACTA guidelines recommend to implement a patient blood management protocol (class I, level of evidence C). Additionally, the use of perioperative POC tests should be considered to reduce the transfusion requirements (class IIa, level B). The STS/ACS guidelines also mention that it is not clear whether the algorithms guiding transfusion and the multidisciplinary approach are more important than the POC testing. Thus, available evidence supports both the use of transfusion algorithms and POC assays to guide transfusion practice and improve blood conservation.

Platelet function tests in cardiac surgery

Furthermore, it was noted that most of the published studies included only viscoelastic coagulation tests, although a specific platelet function test was included in the algorithm in 2 of the more recent studies.(42, 43) Additionally, a systematic review and meta-analysis observed that POC platelet function tests can indeed detect platelet dysfunction in cardiac surgery patients. And that the platelet function tests showed significant variability in their ability to predict blood loss and transfusion requirements. According to Bolliger et al the low predictive value can partly be explained by the fact that the etiology of bleeding after cardiac surgery is commonly multifactorial.(44)

Moreover, the implementation of a platelet function test into a blood management protocol was associated with reduced blood loss and red blood cell and plasma transfusion demands. The review also states that the combination of viscoelastic and other platelet function testing methods achieved a larger effect size in terms of blood loss reduction than viscoelastic methods alone. Also, it was shown that the combination of viscoelastic and platelet function testing results in less red blood cell transfusions than with viscoelastic testing alone. However, for platelet transfusions it was the opposite: viscoelastic testing alone resulted in less platelet transfusions than the combination of viscoelastic and platelet function testing.(45) A more recent narrative review about POC platelet function testing stated that for clinical use, cut-off values to define high postoperative bleeding risk or increased risk for ischemic events definitively need to be

better validated. In addition, it concludes that studies evaluating the value of platelet function tests in clinical decisions in bleeding patients after cardiac surgery are sparse. Thus, this review does not yet recommend a common and widespread use of perioperative platelet function testing.

Concluding, it is not undisputed whether POC platelet function tests should be part of blood conservation and transfusion algorithms / protocols. Currently there are a couple of POC platelet function tests that are fast and affordable,(46) but both the international guidelines and the earlier mentioned review papers cannot recommend a specific platelet function test. Future research should determine whether one platelet function POC test is preferred over the others.

On top of that clinically relevant cut-off values have to be established for cardiac surgery patients. The PLATFORM study found that platelet function tests with multiple electrode aggregometry after cardiopulmonary bypass are significantly associated with postoperative bleeding. The study also showed that the ADP-test had the best discrimination and which cut-off value had the best predictive value for postoperative blood loss. (29) More of these type of studies are needed to establish the most accurate platelet function test and the best cut-off values. Once proper cut-off levels for non-acceptable intraoperative and postoperative platelet function are established, POC platelet function tests can actually be added to guidelines, protocols and daily clinical practice.

Another important aspect that needs attention is the dosing of platelet transfusions. In an *ex vivo* study exploring how many platelet units are necessary to undo the clopidogrel effect, the equivalent of five units, was not even sufficient to achieve normal platelet aggregation under flow conditions.(47) As *in vitro* platelet aggregation is strongly correlated with postoperative hemorrhage it is possible that the finding of this study indicates that transfusion with one unit of platelets for patients on antiplatelet drugs is insufficient to correct the bleeding disorder. Future research is required to evaluate the optimal dose of platelet units in certain clinical situations. Concurrently, obviously surgical techniques and surgical tools that reduce the surgical tissue injury and thereby the need for transfusions should continuously be developed and improved.

Impact of storage time on safety and efficacy

In our study in chapter 6 we evaluated in cardiac surgery patients whether storage time of transfused platelet concentrates was associated with adverse outcomes. The most important finding of this study was that, in our cardiac surgery population, transfusion of old platelets was associated with a higher in-hospital mortality when compared to transfusion of fresh platelets. Moreover, patients transfused with old platelets more often suffered from blood loss of 1000 mL or more (in the first 12 hours after surgery)

and more often required reoperation for bleeding than patients transfused with fresh platelets.

We selected cardiac surgery patients who received solely old platelets or solely fresh platelets. That enabled a clear comparison without the influence of exposure to both types of platelets. Differences in outcomes between patients exposed to platelets with different storage time may have been due to other risk factors for the outcome. Yet, clinical decisions to transfuse fresh or old platelet concentrates were made independently from the storage time of the platelet concentrates, as the treating physicians was not aware of and could not influence the storage time of the transfused concentrates. Platelets are issued according to the first-in-first-out principle. Therefore, the storage time can be influenced by the number of platelets a patient receives, because the more concentrates a patient receives, the bigger the chance that that patient will receive a fresh concentrate (as these concentrates lie in the back of the shelf). Therefore, patients with unfavorable risk profiles and worse prognosis tend to receive more platelet concentrates with a higher chance of receiving fresher platelets. To minimize this potentially confounding factor of the amount of platelet transfusions, our analyses were corrected for number of platelet transfusions administered. Even if residual confounding is left despite the correction, then we expect it to result in an underestimation of the actual effect, because the patients with a less favorable prognosis received younger platelets then the patients with a better prognosis. The analyses were also corrected for logistic EuroSCORE, age, gender, left ventricular function, recent myocardial infarction, isolated CABG, and hospital. EuroSCORE is an internationally accepted scoring system for the prediction of early mortality in cardiac surgical patients (in Europe) on the basis of objective preoperative risk factors.(48) So by correcting for EuroSCORE factors that are considered relevant for early mortality in cardiac surgery are taken into account. We therefore conclude that it is unlikely that bias or confounding explain our findings.

To the best of our knowledge there is only one other study that investigated the association between storage time of platelets and clinical outcomes of cardiac surgery patients. (49) In contrast to our study, this study did not find an association between storage time of platelets and post-cardiac surgery adverse outcomes. A possible explanation for the difference between the findings is that the study of Welsby et al only contained nonemergent CABG patients transfused with apheresis platelets with a maximum storage of 5 days while our study included both elective and non-elective cardiac surgery patients transfused with pooled buffy-coat platelets with a maximum storage of 7 days. So our database contains more high-risk patients who might also be more vulnerable for the potential influence of transfusion with old platelets. This difference in risk-profile of the patient populations is also reflected in the hospital mortality (of 5.2%) observed by Welsby et al, that is lower than the hospital mortality we observed (9.0%). Besides,

possibly the "platelet storage lesion" is different in apheresis than in pooled platelets and more importantly the two days longer maximal storage time in our pooled platelets might lead to more "storage lesion". Our main analysis contained a larger population and more importantly our population consisted of patients transfused solely with old or solely with fresh platelets to avoid dilution of the association.

Our findings suggest that in-hospital mortality, postoperative (first 12 hours) blood loss >1000 mL and reoperation for bleeding occur more frequently following transfusion of old platelets. Based on these results the most obvious potential explanation for the higher hospital mortality are the higher blood loss and the resulting higher need for reoperation. If this is the actual explanation for the higher hospital mortality after transfusion with old platelets, this would suggest that old platelets are less effective in preventing and/or stopping bleeding in cardiac surgery. This possible explanation for our findings is plausible in the light of the discoveries earlier studies showed: the increase of platelet activation, while responsiveness to agonist decreased and a decline of platelet viability and function, together called the "platelet storage lesion".(5, 15, 50-54) Besides the possibly reduced efficacy of old platelets compared to fresh platelets, other possible (partial) explanations of the higher in-hospital mortality could be a higher risk of TRALI, other transfusion reactions or other complications that was observed with longer storage of platelets.(55-58)

The possible clinical implication of these results is that cardiac surgery patients are transfused with fresher platelet products as compared with current practice. Yet, this is just one study, with a study design that is suboptimal with respect to minimization of bias. Therefore, we feel that future studies should first confirm or refute the findings of the current study. In conclusion, in our cardiac surgery population transfusion of old platelets was associated with a higher hospital mortality, more blood loss and more reoperations for bleeding compared to fresh platelets. In our opinion these results instigate further research into this topic because if old platelets actually do cause higher hospital mortality in cardiac surgery patients this asks for a change in clinical practice.

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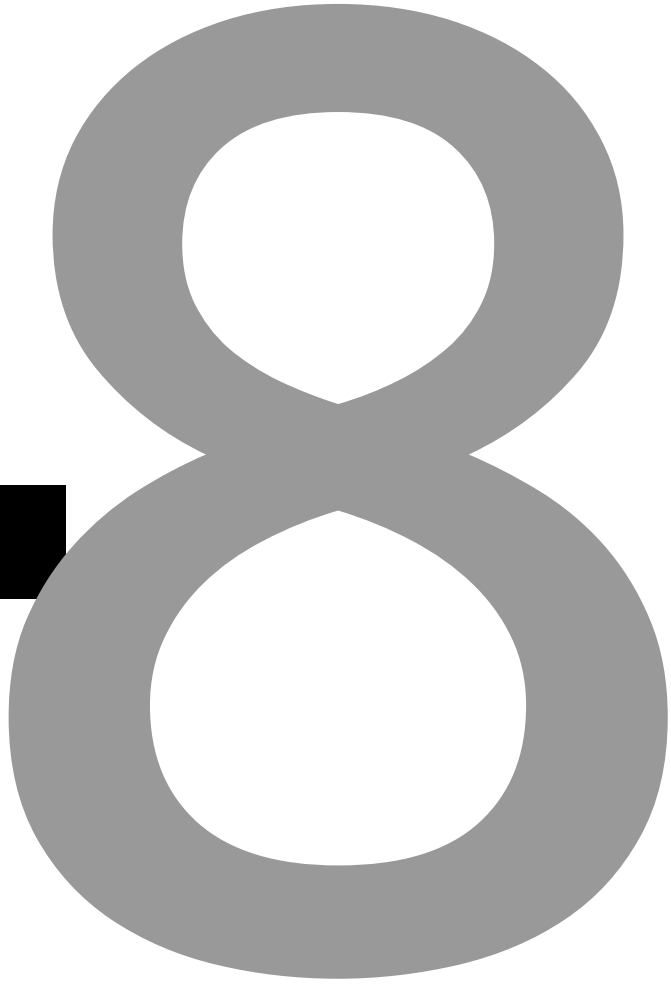
CHAPTER 8



Summary

Platelet transfusions are used to provide hemostatic capacity to patients with decreased number or functionality of platelets. The aim of this thesis was to expand knowledge about the safety and efficacy of platelet transfusions in general and in particular in cardiac surgery patients. In the decision to transfuse a patient with platelets, like in every clinical decision, it is important to weigh the potential benefits against the potential harm this treatment can cause. Therefore, we aimed at gaining more knowledge about potential harms and benefits for recipients of a platelet concentrate, and the storage medium and storage time of platelet concentrates. Expansion of knowledge about this topic can help clinicians to decide which patients to transfuse with platelets at which moment and to create possibilities to improve the safety and efficacy of platelet transfusions.

In the Netherlands most platelet concentrates are prepared using five buffy coats from whole blood donations (pooled platelets concentrates). These buffy coats are then resuspended either in 30-40% plasma and 60-70% platelet additive solution (PAS) (PAS-platelets) or, until April 2018, in 100% plasma of one of the five donors (plasma-platelets). In hematology patients, it has been described that reducing the amount of plasma in platelet units lowers the incidence of transfusion reactions. However, the results of these studies may not be applicable to other patients receiving platelet transfusions, like trauma or cardiac surgery patients. In our nationwide study, in **chapter 2**, covering a 10-year period with more than half a million platelet transfusions, we showed that PAS-C-platelets are associated with less transfusion reactions than plasma-platelets and PAS-B platelets.

Besides safety also efficacy is important to consider when comparing PAS-platelets with plasma-platelets. It is unclear whether PAS-platelet concentrates are as effective as plasma-platelet concentrates. A previous *in vitro* study, comparing platelets stored in different types of PAS with plasma-platelets, demonstrated inferior results for PAS-platelets compared to plasma-platelets. However, in this study testing was performed in the absence of red blood cells and blood plasma. Moreover, the correlation between the analyzed in-vitro endpoints and clinically relevant endpoints has not been established. The clinical studies analyzing the effectiveness of PAS-platelet transfusions have been performed in hematologic patients and have shown conflicting results. The results of these previous studies are possibly not applicable to surgical and trauma patients. In our *in vitro* study, we compared the hemostatic function of PAS-platelets to plasma-platelets in reconstituted whole blood using Multiplate-derived platelet aggregation, thromboelastography-measured overall clot formation and CD62P responsiveness. These tests are reported to correlate well with clinically relevant outcomes. As described

in **chapter 3**, we observed that the (Multiplate-derived) aggregation and the agonist induced CD62P responsiveness of PAS-C-platelets was lower than that of plasma-platelets. However, TEG-derived clot formation, showed no differences between the PAS-C-platelets and plasma-platelets.

Besides storage medium also the storage time of platelet concentrates is of interest for the safety and efficacy of platelet transfusions. Storage time has been associated with the accumulation of biological response modifiers, such as inflammatory cytokines and chemokines. Whether these changes have clinical consequences is not clear yet, as published results are contradictory. In our study, in **chapter 4**, we demonstrated that longer storage time is associated with a higher incidence of transfusion reactions in PAS-platelets. In plasma-platelets storage time showed no association with transfusion reaction rates. However, the overall incidence of transfusion reactions, so regardless of storage time, following plasma-platelets is higher than that of PAS-C-platelets. In our *in vitro* study, in **chapter 3**, (Multiplate-derived) aggregation and (TEG-derived) initial clot formation significantly declined with increasing storage time both in plasma-platelets and in PAS-platelets. Yet, (TEG-derived) maximum clot strength and clot growth rate did not decline with increasing storage time.

In addition to the characteristics of the platelet concentrate it is likely that patient characteristics and the specific clinical situation influence the efficacy of a platelet transfusion. Since a significant part of platelet transfusions is consumed by cardiac surgery patients, it is important to understand the impact of a platelet concentrate and its storage conditions on cardiac surgery patients. There is a lack of clinical evidence establishing the hemostatic effect of platelet transfusions in cardiac surgery patients. In addition, conflicting results have been reported regarding the safety of platelet transfusions in the setting of cardiac surgery. So, there is an unmet need to determine the clinical impact (safety and efficacy) of perioperative platelet transfusions in patients undergoing cardiac surgery. To the best of our knowledge, our study in **chapter 5** was the first describing not only the adverse outcomes, but also the intended effects of a platelet transfusion in cardiac surgery patients and thereby presenting an overall picture of the outcomes following platelet transfusion. We selected patients who received one platelet concentrate (and no other blood products) shortly after cardiopulmonary bypass (CPB). These patients were matched 1:3, based on propensity score, to patients who did not receive any blood products shortly after CPB. In this retrospective analysis, cardiac surgery patients receiving platelet transfusion in the operating room experienced less blood loss, but more often required vasoactive medication, prolonged ventilation, prolonged intensive care admission, and transfusion of other blood products postoperatively. Moreover, early platelet transfusion was not associated with reinterventions, thromboembolic complications, infections, multi-organ failure, or mortality.

During storage platelets undergo multiple changes in structure and function collectively known as the "platelet storage lesion". It is conceivable that in patients this "platelet storage lesion" results in a reduced hemostatic capacity and more adverse events. Subsequently, the question arose whether these "platelet storage lesions" have clinical consequences. In our study, described in **chapter 6**, in cardiac surgery patients we found that transfusion of old platelets was associated with a higher in-hospital mortality when compared to transfusion of fresher platelets. Moreover, patients transfused with old platelets more often suffered from blood loss of 1000 mL or more (in the first 12 hours after surgery) and more often required reoperation for bleeding than patients transfused with fresh platelets.

In **chapter 7** the main conclusions of this thesis are described and discussed. In this thesis we concluded that cardiac surgery patients receiving an early platelet transfusion experienced less blood loss than patients who did not receive an early platelet transfusion. However, early platelet transfusion was not associated with more reinterventions, thromboembolic complications, infections, multi-organ failure, or mortality. Furthermore, we concluded that PAS-C as storage medium for platelets is associated with more favorable outcomes than plasma with regard to transfusions reactions, but not with regard to *in vitro* hemostatic measurements. Furthermore, we concluded that fresh platelet concentrates are associated with more favorable outcomes than older platelet concentrates: less transfusions reactions in the whole transfused population, better hemostatic measurements *in vitro*, and less blood loss and mortality in cardiac surgery patients.

Nederlandse samenvatting

Trombocytentransfusies worden gegeven aan patiënten die te weinig eigen trombocyten hebben of wanneer de eigen trombocyten niet goed functioneren om bloedingen te stelpen of te voorkomen. Het doel van dit proefschrift was om de kennis te vergroten over de effectiviteit en veiligheid van trombocytentransfusies in het algemeen en in het bijzonder bij hartchirurgische patiënten. Net als voor iedere klinische beslissing is het voor de beslissing om een patiënt een trombocytentransfusie te geven nodig om een afweging te maken tussen wat het de patiënt op kan leveren en hoe het de patiënt kan schaden. Wij onderzochten niet alleen de effectiviteit en veiligheid van trombocytentransfusies zelf, maar ook de invloed van de bewaarvloeistof en de bewaarduur daarop. Kennis hierover kan clinici helpen bij de beslissing welke patiënten zij op welk moment al dan niet een trombocytentransfusie geven. Aldus creëert meer kennis de mogelijkheid om de effectiviteit en veiligheid van trombocytentranfusies te optimaliseren.

In Nederland worden de meeste trombocytenconcentraten samengesteld uit de "buffy coats" van vijf volbloeddonaties. Deze vijf "buffy coats" werden, tot april 2018, bewaard in het plasma van een van die vijf donoren (plasma-trombocyten) óf in 65% "platelet additive solution" (PAS) en 35% plasma (PAS-trombocyten).

In eerdere studies met hematologische patiënten is beschreven dat de incidentie van transfusiereacties daalt wanneer de hoeveelheid bloedplasma in trombocytenconcentraten verlaagd wordt. Het was nog niet bekend of deze bevindingen ook gelden voor andere patiëntpopulaties die trombocytentransfusies ontvangen zoals traumapatiënten en hartchirurgische patiënten. Om dit te onderzoeken hebben wij een nationale studie gedaan, beschreven in **hoofdstuk 2,** over een periode van 10 jaar waarin meer dan een half miljoen trombocytentransfusies hebben plaatsgevonden. In deze studie vonden we minder transfusiereacties na toediening van PAS-C-trombocyten dan na plasma-trombocyten en PAS-B-trombocyten.

Bij het vergelijken van plasma-trombocyten met PAS-trombocyten is naast de veiligheid ook de effectiviteit belangrijk. In een *in vitro* studie lieten PAS-trombocyten slechtere uitkomsten zien dan plasma-trombocyten. Echter, in die studie werden de trombocytenconcentraten met elkaar vergeleken in de afwezigheid van de andere componenten van bloed. Wij hebben in onze *in vitro* studie in **hoofdstuk 3** de hemostatische functie van PAS-trombocyten vergeleken met plasma-trombocyten in gereconstitueerd volbloed. De vergelijking werd gemaakt aan de hand van drie tests waarvan een goede correlatie met klinische uitkomsten is beschreven: trombocytenaggregatie gemeten met de Multiplate, de bloedstolselvorming gemeten met tromboelastografie (TEG) en de agonist-geindiceerde CD62P responsiviteit. In het gereconstitueerde volbloed met

PAS-C-trombocyten was de gemeten tromboytenaggregatie (Multiplate) en de agonist-geïndiceerde CD62P responsiviteit lager dan bij plasma-trombocyten. Echter, er was geen verschil tussen het gereconstitueerde bloed met de PAS-C-trombocyten en met de plasma-trombocyten wat betreft de stolselvorming (TEG).

Naast de bewaarvloeistof van de trombocyten is ook de bewaarduur mogelijk van invloed op de effectiviteit en de veiligheid van trombocytentransfusies. Uit eerdere studies is gebleken dat gedurende het bewaren van de trombocyten zich stoffen ophopen in het trombocytenconcentraat, de zogenaamde "biological response modifiers" zoals inflammatoire cytokines en chemokines. Of en hoe deze "biological response modifiers" gevolgen hebben voor de patiënt was nog niet bekend. In onze studie in **hoofdstuk 4** hebben wij laten zien dat in PAS-trombocyten een langere bewaarduur geassocieerd is met meer transfusiereacties. Terwijl in plasma-trombocyten de bewaarduur niet geassocieerd was met meer transfusiereacties. Nota bene, de incidentie van transfusiereacties is over het algemeen, dus los van bewaarduur, hoger na plasma-trombocyten dan na PAS-C-trombocyten.

In onze *in vitro* studie in **hoofdstuk 3**, zagen we dat de aggregatie (gemeten met Multiplate) en initiële stolselvorming (gemeten met TEG) significant afnam naarmate bewaarduur van de PAS- en plasma-trombocyten toenam. Echter de maximale stevigheid en de ontstaanssnelheid van het stolsel (TEG) namen niet af bij langere bewaarduur.

Naast de karakteristieken van het trombocytenconcentraat is het ook aannemelijk dat de eigenschappen van de patiënt en de specifieke klinische situatie invloed hebben of het effect van een transfusie. Aangezien een aanzienlijk deel van de trombocytentransfusies aan hartchirurgische patiënten wordt gegeven, is het belangrijk te weten hoe de effectiviteit en de veiligheid van een trombocytentransfusie is in deze populatie.

Er is geen bewijs uit klinische studies of en hoe effectief een trombocytentransfusie is op de hemostatische situatie van deze patiënten. Bovendien zijn er tegenstrijdige bevindingen in eerdere studies wat betreft de nadelige effecten die een trombocytentransfusie kan hebben. Voor zover wij weten was onze studie in **hoofdstuk 5** de eerste die zowel het beoogde hemostatische effect als de ongunstige uitkomsten van trombocytenconcentraten bij hartchirurgische patiënten onderzocht en daarmee een overzicht van de uitkomsten na een trombocytentransfusie schetst. De patiënten die in de operatiekamer kort na de ingreep (een vroege) trombocytentransfusie ontvingen, hadden daarna minder bloedverlies, maar vaker vasoactieve medicatie, langdurige beademing, en langdurige IC opname en kregen vaker andere bloedproducten, vergeleken met patiënten die geen vroege trombocytentransfusie ontvingen. Het aan-

tal reinterventies, trombo-embolische complicaties, infecties, multi-orgaan falen en de mortaliteit verschilde niet tussen de beide groepen.

Gedurende het bewaren ondergaan de trombocyten in een trombocytenconcentraat meerdere veranderingen in structuur en functie die samen de "platelet storage lesions" genoemd worden. Of deze "platelet storage lesions" leiden tot verminderde hemostatische effectiviteit en of ze gepaard gaan met nadelige klinische uitkomsten was nog onduidelijk. In **hoofdstuk 6** beschrijven we dat in onze hartchirurgische studiepopulatie transfusie van relatief oudere trombocyten geassocieerd was met een hogere ziekenhuismortaliteit dan transfusie van versere trombocyten. Bovendien, zagen we na transfusies van oudere trombocyten concentraten dat er vaker sprake wasvan meer dan 1000 mL bloedverlies (binnen 12 uur na de operatie) dan na transfusie van versere trombocyten.

In **hoofdstuk 7** worden de belangrijkste conclusies van dit proefschrift beschreven en bediscussieerd. In dit proefschrift concludeerden we dat hartchirurgische patiënten die kort na de ingreep een trombocytentranfusie kregen minder bloed verloren dan patiënten die dat niet kregen, maar dat het aantal reinterventies en de mortaliteit niet verschilden tussen de groepen. Daarnaast was een trombocytentransfusie kort na de chirurgische ingreep niet geassocieerd met trombo-embolische complicaties, infecties of multi-orgaan falen. We lieten zien dat de bewaarvloeistof PAS-C geassocieerd was met gunstigere uitkomsten dan plasma wat betreft transfusiereacties, maar niet wat betreft *in vitro* hemostatische parameters. Ook toonden we aan dat transfusie van versere trombocytenconcentraten geassocieerd was met gunstigere uitkomsten dan van oudere concentraten: minder transfusiereacties in de gehele getransfundeerde populatie, betere hemostatische parameters *in vitro*, en minder bloedverlies en mortaliteit in hartchirurgische patiënten.

Curriculum Vitae

Fabienne Maria Antonia Plucinski - van Hout werd geboren in Nijmegen op 4 mei 1987. Na een korte periode in Nijmegen, groeide zij de eerste 10 jaar op in Boskoop waarna zij met haar ouders verhuisde naar Nootdorp. Vanuit daar startte zij met het voortgezet onderwijs op het Stanislas College in Delft. In de herfst van 2000 verhuisde zij met haar ouders, broertje en zusje naar Spijkenisse waar zij verder ging met het gymnasium op de locatie Blaise Pascal van CSG Penta College. In 2005 ronde zij het atheneum cum laude af waardoor zij aan de Universiteit Leiden, haar eerste keuze, aan de studie geneeskunde kon beginnen. Al aan het begin van de studie werd haar interesse gewekt voor de snijdende vakken en in het bijzonder voor de cardiothoracale chirurgie. In de loop van haar studie had zij verschillende bijbaantjes, onder andere als student-assistent bij de snijzaal anatomie practica. Ondanks dat onderwijs geven ook voor wat nervositeit zorgde, vond zij het fantastisch en raakte nog enthousiaster over het menselijk lichaam, de anatomie ervan en de snijdende vakken. Tijdens haar wetenschapsstage deed ze onderzoek naar asymptomatische mitralisklepinsufficiëntie bij de afdeling cardiothoracale chirurgie van het LUMC onder begeleiding van prof. dr. Robert Klautz en dr. Meindert Palmen. Ze mocht dit onderzoek in 2010 presenteren bij de Nederlandse Vereniging van Thoraxchirurgie en in 2013 op de AATS Mitral Conclave in New York en op het SHVD & HVSA congres in Venetië. Na haar wetenschapsstage en het doorlopen van de coschappen sloot zij in 2012 haar studie af met haar semi-artsstage bij de cardiothoracale chirurgie van het LUMC. Na afronding van de studie geneeskunde begon zij gelijk als ANIOS cardiothoracale chirurgie in het LUMC, om na een klein jaar te starten met haar promotietraject onder begeleiding van prof. dr. Anske van der Bom, dr. Jean-Louis Kerkhoffs en dr. Meindert Palmen. Dit onderzoek richtte zich op de effectiviteit en veiligheid van trombocytentransfusies in het algemeen en in het bijzonder bij hartchirurgische patiënten. De resultaten van dit promotie onderzoek staan beschreven in dit proefschrift, heeft zij gepresenteerd op diverse nationale en internationale congressen en zijn gepubliceerd in internationale wetenschappelijke tijdschriften. In de loop van haar promotietraject realiseerde zij zich dat oogheelkunde beter bij haar en haar leven past dan cardiothoracale chirurgie. Vandaar dat zij na haar promotietraject als ANIOS ging werken bij de afdeling Oogheelkunde van het Haga Ziekenhuis. Een klein jaar later mocht zij in 2018 starten met de opleiding tot oogarts in het Erasmus MC te Rotterdam die zij in 2023 zal afronden. In Rotterdam leerde Fabienne de liefde van haar leven, Daan Plucinski - van Hout, kennen. Zij kregen op 17 september 2022 hun geweldige zoon Rogier.

List of publications

van Hout FMA, Hogervorst EK, Rosseel PM, van der Bom JG, Bentala M, van Dorp EL, van Geloven N, Brand A, van der Meer NJ, van de Watering LM. Does a Platelet Transfusion Independently Affect Bleeding and Adverse Outcomes in Cardiac Surgery? Anesthesiology. 2017 Mar;126(3):441-449.

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