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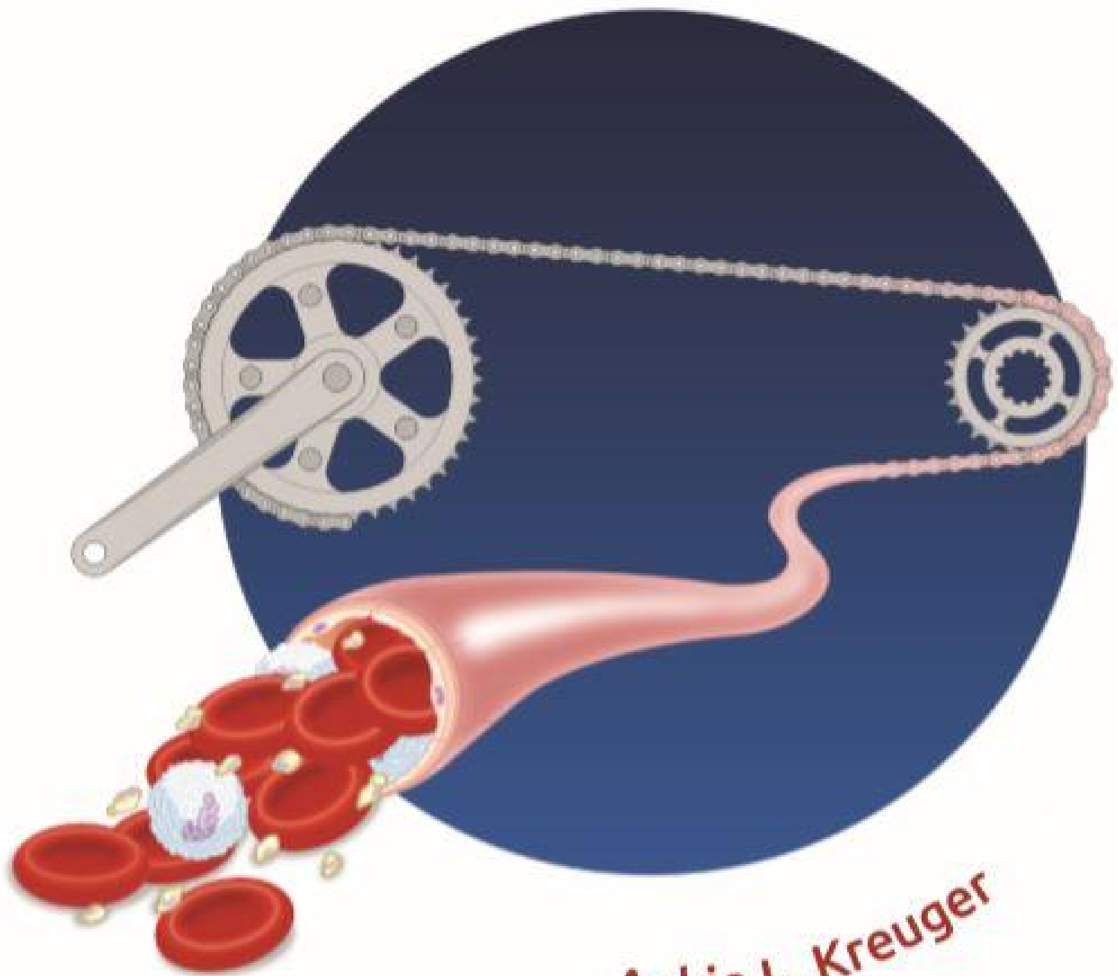
Platelet transfusions in patients with a hematological malignancy

Improving the chain



Aukje L. Kreuger

Platelet transfusions in patients with a hematological malignancy - Improving the chain

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Improving the chain

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Improving the chain

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The work described in this thesis was performed at the Center of Clinical Transfusion Research, Sanquin Research, Leiden, and the department of Clinical Epidemiology, Leiden University Medical Center, Leiden.

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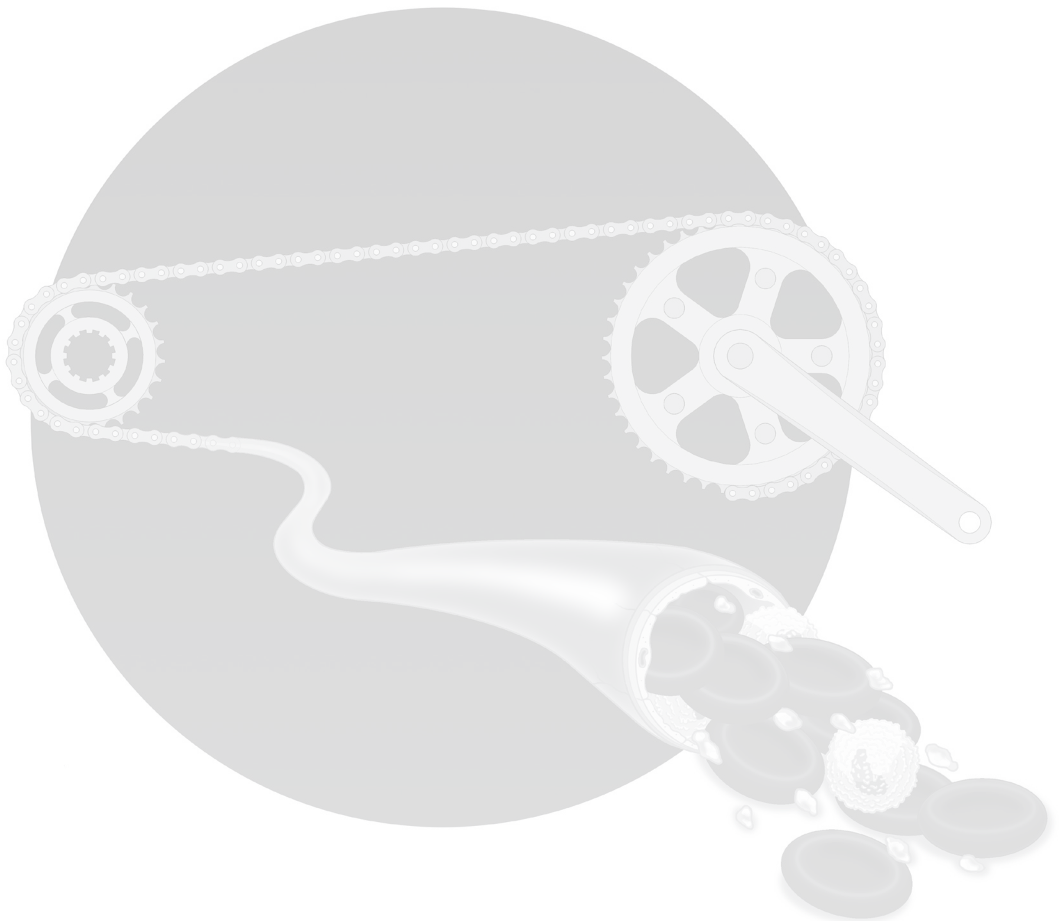

Chapter 1

General introduction and outline of this thesis

A healthy individual has approximately 150-400x10^{9} platelets per liter in the circulation. In patients with a hematological malignancy this can drop to unmeasurable low amounts as a consequence of the treatment or the disease itself, leading to an increased risk of hemorrhage. These patients need platelet transfusions to prevent or treat these hemorrhages. Yearly, approximately 59,000 platelet concentrates are transfused in the Netherlands, 2.9 million in Europe and 1.5 million in the USA, of which the majority, up to 67%, are given to hematological patients.[1-5]

History

The history of platelet transfusions goes back to the beginning of the twentieth century. In 1910, William Duke demonstrated the role of platelets in stopping hemorrhages and the beneficial effect of transfusions on platelet count, bleeding time and bleeding tendency. Duke described three patients with spontaneous hemorrhages in whom the bleeding time normalized after a transfusion of whole blood and in two of these patients the bleeding even stopped. This resulted in the suggestion that a low platelet count could be a cause of hemorrhages instead of only an accompanying symptom.[6] Nowadays, Duke's paper is known as a landmark paper and one of the outstanding contributions to medicine in the first half of the twentieth century.[7]

It took until 1962 before Gaydos et al. quantified the relationship between platelet count and the occurrence and severity of hemorrhages. Hardly any patient bled at a platelet count above 20x10^{9} platelets/L.[8] Although Gaydos et al. did not define a transfusion threshold, this study and two small trials laid the groundwork for prophylactic platelet transfusions at a trigger of 20x10^{9} platelets/L.[9,10] This became an essential part of supportive care for thrombocytopenic patients and was considered to be the leading reason for the major decline of fatal hemorrhages among leukemia patients from 67% to 37% in the sixties.[11,12] The increased demand for platelet concentrates and the concerns about the risks and costs of transfusions resulted in several studies comparing the efficacy of different thresholds for platelet transfusions.[13-19]

Transfusion practice

Nowadays, a prophylactic transfusion policy with a trigger of 10x10^{9} platelets/L is recommended for clinically stable patients.[20-23] The safety of this threshold is supported by the suggestion that a platelet count of 7.1x10^{9} per liter per day is sufficient to maintain vascular integrity.[24]

The alternative to maintaining the platelet count above certain threshold with a prophylactic transfusion policy is a therapeutic policy in which patients only receive platelets in case of a symptomatic bleeding. Although such a policy results in a reduction of the total number of transfused products compared to a prophylactic policy, the risk of bleeding increases and the bleeding free survival shortens, according to a recent Cochrane analysis.[25] Only for patients receiving autologous stem cell transplantation a therapeutic transfusion strategy could be safe.[26,27]

A prophylactic transfusion policy with a trigger of $10x10^9$ platelets/L is probably not sufficient in non-stable patients who face an increased risk of bleeding. Reported risk factors for bleeding are previous bleedings, active infection (predominately fungal infections), fever, allogenic transplantation, graft versus host disease, severe mucositis, and, in older studies, also leukocytosis.[28,29] It is debated which trigger should be adhered by these patients and recommendations in guidelines are based on consensus and differ between countries. The same uncertainty applies to patients undergoing an intervention which potentially increases the bleeding risk. The variation in recommendations results in variation in clinical practice.[30] Although it is unknown whether increasing the threshold truly reduces the bleeding risk, many clinicians increase the transfusion threshold to counterbalance the assumed increased risk of bleeding.

Efficacy of platelet transfusions

The main reason to transfuse platelets is to prevent or treat hemorrhages. Of all platelet transfusions issued to hematological patients, 69% is given for prophylactic purposes to patients with a platelet count $<10x10^9$ platelets/L.[3] The clinically most relevant outcome to evaluate the efficacy of these transfusions is the incidence of bleeding. Severity of bleeding can be categorized according to the WHO grading scale.[31] The original scale has been adapted to make it less prone to subjective interpretation and suitable to use in several trials. Table 1 shows the scale with the definitions as has been used in the PlaDo and PREPAReS trials.[32,33] The TOPPS trial used a slightly different scale with as main difference the classification of 'CNS bleeding noted on CT scan without symptoms' as grade 3 instead of grade 4 hemorrhage.[27]

Table 1. WHO bleeding severity score[32,33]

Grade	Symptoms
Grade 1: Minor	Petechiae, oropharyngeal bleeding Epistaxis <30 minutes Purpura <1 inch Occult blood stool (1+) Urine hemoglobin (1+) Vaginal bleeding, spotting
Grade 2: Mild blood loss	Melena, hematemesis, hemoptysis, hematuria, hematochezia, abnormal vaginal bleeding, not requiring RBC transfusion Epistaxis, oropharyngeal bleeding > 30 minutes Retinal hemorrhage without visual impairment Occult blood stool (≥2+) Urine hemoglobin (≥2+) Abnormal vaginal bleeding, more than spotting
Grade 3: Gross blood loss	Any bleeding requiring RBC transfusion over routine transfusion needs Bleeding from invasive sites
Grade 4: Debilitating blood loss	Debilitating bleeding including retinal bleeding with visual impairment CNS bleeding Bleeding associated with hemodynamic instability Fatal bleeding

The use of bleeding as main outcome measure in studies regarding the efficacy of platelet transfusions is challenging. Documentation of signs and symptoms of bleeding is labor intensive and it is difficult to translate these into a single score.[34] Differences in observation methods and grading systems resulted in large variation in reported incidences.[35] Moreover, the clinically most relevant bleedings, grade 3 and 4, have a low incidence and therefore large sample sizes would be required to obtain sufficient power.[36]

An alternative measure of efficacy of platelet transfusions is a platelet count increment, which can be measured after each transfusion. 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, by using the following formula:

$$CCI = \frac{(post\ transfusion\ platelet\ count(10^9) - pretransfusion\ platelet\ count(10^9))\ x\ body\ surface\ area\ (m^2)}{platelets\ transfused\ (10^{11})}$$

A standard platelet concentrate in the Netherlands contains on average $380 \pm 55 \times 10^9$ platelets.[33] The CCI is usually calculated 1 or 18-24 hours after transfusion. The 1 hour CCI is predominantly determined by quality of the product, spleen size, and alloimmunization, whereas the 24 hours CCI expresses the survival of platelets and is mainly influenced by the clinical condition of the patient.[20,37] A patient is refractory to platelet transfusions when two subsequent fresh ABO identical transfusions are unsuccessful. According to the Dutch CBO guideline, a transfusion is unsuccessful when the 1 hour CCI is <7.5 dm^{-1} or the 24 hours CCI <4.5 dm^{-1}.[20]

Transfusion side-effects

As applies to everything in medicine, also transfusions are not without side effects. A quote attributed to Karl Landsteiner (1868-1943), who described the AB0 blood group system as first, stated "A blood transfusion should never be ordered or given unless it is worth the risk".[38,39] This quote is still valid, although many improvements have been made since.

Nowadays, the risk of dying as a direct consequence of a transfusion has been estimated to be around 1 in 322,580 and the risk of major morbidity around 1 in 21,413 transfused components.[40] Transfusion reactions vary from mild urticaria to severe transfusion reactions or even death. Compared to plasma or red blood cell transfusions, platelet transfusions carry the highest risk of transfusion reactions.[41,42] In the Netherlands, the incidence of severe reactions was 0.18 per 1000 red blood cell transfusions in 2015 compared to 0.38 per 1000 platelet transfusions. Including all severities, these incidences ranged from 4.28/1000 till 5.22/1000 transfusions.[41] One of the most feared adverse reactions is transfusion associated sepsis. If this is directly related to the transfusion of a contaminated product, this is called a transfusion transmitted bacterial infection (TTBI).[41] The incidence of TTBI ranges from 7 up to 26 per million transfused platelet components.[43,44] This variation could be partly explained by differences in vigilance of reporting, but also differences in products could play a role.

The platelet concentrate

Internationally, large variation exists in methods to collect, produce and store platelets. In the Netherlands, 90 to 95% of platelet concentrates are prepared from buffy coats and the remaining 5 to 10% of issued platelet concentrates are derived via apheresis. These are only used for specific indications like neonates, or refractory

patients who need HLA or HPA-matched platelets. For the buffy coat method, whole blood is held overnight at room temperature and split by hard spin centrifugation into a red cell layer, plasma, and the buffy coat, consisting of platelets and leukocytes. Buffy coats of five donors with the same AB0 and rhesus D blood group are pooled, leukocytes are removed via a soft-spin procedure and filtration, and the platelets are resuspended in plasma or platelet additive solution (PAS), with 25ml of plasma left per donor.[20]

Storage medium

PAS is a generic term for a solution with a standardized composition of electrolytes. It was developed in the 1980s to remove plasma from the platelet concentrate, as it was thought that plasma had a deleterious effect on platelet quality during storage. Other supposed advantages of PAS were a reduced risk of allergic reactions, a lowered anti-AB0-titer and the conservation of plasma for fractionation.[2,45]
PAS gave the opportunity to control the storage environment. Most important is to maintain a pH above 6.0 to maintain platelet viability. The main energy source of platelets is oxidation of glucose into ATP and lactic acid, resulting in lowering of the pH, which in turn leads to more activation of platelets and thereby more glucose consumption and accompanying lactic acid production, a vicious circle. Most PASs contain acetate as nutrient for platelets to hamper 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 PASs still contain 20-35% of plasma as main source of glucose and to maintain platelet membrane integrity.[2,46,47]

In the Netherlands, both plasma and PAS are used as storage medium. The geographic location of the hospital determines the choice of storage medium. In hospitals in the South-West of the Netherlands PAS is used, whereas in the other regions plasma is used as main storage medium.[2] PAS-B (T-sol, Baxter) was used up to 2012, and PAS-C (Intersol, Fenwal, Inc) since January 2013. The difference between PAS-B and PAS-C is the addition of phosphate as extra buffer in PAS-C. From January 2018 PAS-E will be used as additive solution.

Storage time

Within the circulation, platelets can survive up to ten days. This shortens to as low as 3.4 days when the platelet count drops below $20x10^9$/L.[24] Once donated, platelets can be stored for up to seven days at room temperature under constant agitation. As these are ideal conditions for bacterial proliferation, all products are screened for bacterial contamination. In the Netherlands, the BacT/Alert system (bioMérieux, Nürtingen, Germany) is used, consisting of an aerobic and an anaerobic bottle, each inoculated with 7.5 ml of the platelet product. Products are released according to a 'negative to date' system, which means that products can be transfused as long as the BacT/Alert stays negative.[48] Internationally, large variation exists in the maximal allowed storage time of platelet concentrates. Besides in the Netherlands, storage up to seven days is also allowed in Spain and Denmark, whereas this is limited to 5 days in Canada, Austria and the United States. Without bacterial screening, storage is limited to 3.5 days in Japan, 4 days in Germany and to 5 days in France.[49] As outdating is the main reason for discarding of platelet concentrates, prolonged storage could have logistic benefits.[50] However, *in vitro* studies showed a gradual loss of platelet function during storage.[51] These 'platelet storage lesions' could also implicate a loss of hemostatic functions *in vivo*.[52]

Transfusion research

In general, transfusions are safe, effective, and integrated in daily practice. Despite the high quality of supportive care, major hemorrhages do still complicate the treatment of patients with a hematological malignancy. Moreover, adverse events related to the transfusion still occur. This illustrates the importance of studies to improve the safety and effectiveness of blood transfusions.

One of the challenges within the field of transfusion medicine is to set up a study with sufficient power and a clinically relevant endpoint. The low incidence of adverse reactions and major hemorrhages, the most relevant measure of effectiveness of platelet transfusions, oblige researchers to include large populations. Observational studies, using routinely collected health care data can be an appropriate method to obtain sufficient power.

In order to investigate the safety and effectiveness of platelet transfusions, we set up a nationwide cohort study, the ATTACH study, in which we collected and assembled data of platelet transfusions issued between 2005 and 2015 in nine hospitals spread around the Netherlands. For all transfused patients additional information was requested regarding transfusions of other blood products, laboratory measurements, blood cultures, and diagnoses and procedures.

Information regarding characteristics of the transfused product was obtained from Sanquin, the national blood supply. TRIP (Transfusion and Transplantation Reactions In Patients), the national hemovigilance organization, provided information on all reported transfusion reactions related to a platelet transfusion since 2003.

The final database comprised 29,440 patients, who received in total 133,424 platelet transfusions. Of these, 5,583 patients (73,383 transfusions) had a diagnosis of a hematological malignancy or aplastic anemia. Variation in transfusion practice and transfused products, i.e. storage time and storage medium, offers the opportunity to study various aspects of platelet transfusions as has been described in **chapter 4, 7 and 9**.

Aim and outline of this thesis

The aim of this thesis was to address several aspects of platelet transfusions in patients with a hematological malignancy in order to improve the safety and effectiveness.

It starts with the decision when to transfuse. For clinically stable patients, a prophylactic transfusion strategy is well accepted and the trigger of $10x10^9$ platelets/L is uniformly implemented in routine care. However, less consensus exists regarding the optimal transfusion threshold for patients with an increased risk of bleeding or those who need to undergo an intervention. In **chapter 2**, we describe the results of a survey among hematologists in which we asked which trigger they adhere in such situations.

Some patients develop anti-HLA or anti-HPA antibodies and as a consequence they become refractory for platelet transfusions. The best option for these patients to prevent or treat hemorrhages is transfusion of HLA and eventually HPA-matched platelet concentrates. In the study described in **chapter 3** we explored the HLA haplotypes of refractory patients in relation to a population of typed Dutch donors.

Ideally, only those patients who face an increased risk of bleeding receive transfusions. In order to study risk factors for bleeding in large populations, we developed a model to identify leukemia patients with major hemorrhage in routinely collected health care data **(chapter 4)**.

Platelets survive for ten days in the normal circulation, so donated platelets are on average five days old. Subsequently, platelet concentrates can be stored for up to seven days. Storage affects safety and efficacy of the transfused products. In **chapter 5**, we systematically reviewed the existing literature about the effect of storage time on measurements, including (corrected) count increment, recovery, survival and half-life of the platelet after transfusion. Subsequently, in **chapter 6**, we reviewed the literature regarding the effect of storage time on clinical outcomes, including transfusion reactions, complications, length of hospital stay, transfusion interval, transfusion need, bleeding and mortality.

The thrombocytopenia in patients with a hematological malignancy is often accompanied by neutropenia which predisposes these patients to an increased risk of infections. With respect to platelet concentrates, it has been suggested that during storage the risk of infections increases. This could be a direct consequence of

contamination and proliferation of bacteria in the product, or indirectly via modulation of the immune response. In **chapter 7** we investigated the association of storage time of platelet concentrates with all-cause bacteremia the day after transfusion. Based on the results of this study, the question remained whether this effect was similar in PAS stored platelets. Therefore, we used Danish transfusion and microbiology databases to examine whether storage of platelet concentrates in PAS –C for up to six or seven days increases the risk of a positive blood culture at different times after transfusion **(chapter 8)**.

In the Netherlands, the geographic location of the hospital determines whether a patient receives a platelet concentrate stored in plasma or in PAS. In **chapter 9** we investigated the effect of storage medium on the risk of transfusion-transmitted bacterial infections.

In **chapter 10** we discuss the main findings and implications for further research and clinical practice, followed by an English and Dutch summary in **chapter 11.**

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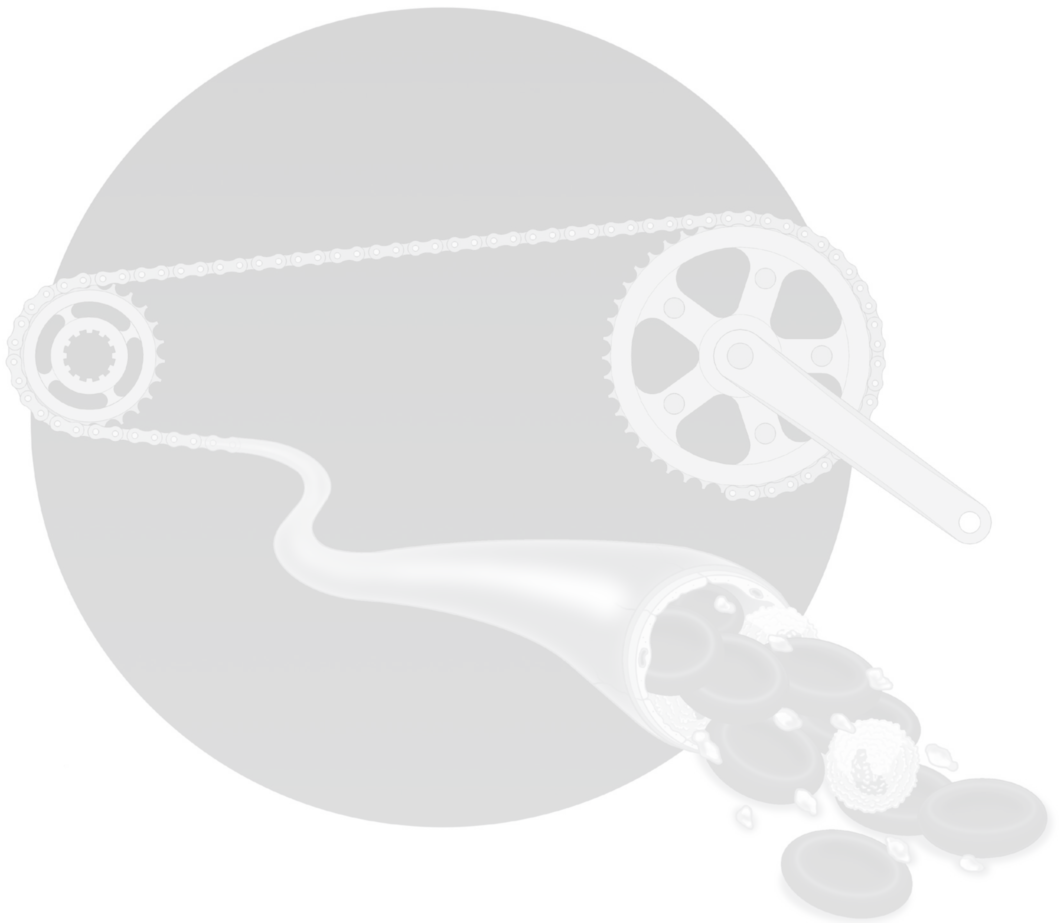

Chapter 2

Clinical practice of platelet transfusions in hemato-oncology

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Abstract

Platelets are prophylactically transfused to patients receiving myeloablative chemotherapy. The trigger can be adapted if a patient has risk factors for bleeding. We performed an international survey to quantify differences in transfusion policies. While platelet counts are most important, bleeding, fever, use of anticoagulants and invasive procedures also determine transfusion strategies. The largest variation of triggers was observed for lumbar punctures and removal of central venous catheters.

Introduction

Patients suffering from hematological malignancies often experience thrombocytopenia. Platelets are routinely administered at a trigger of $10x10^9$/L. However, this does not prevent bleeding in all patients. Uremia, hypoalbuminea, recent bone marrow transplantation, recent bleeding, fever, and use of anticoagulation are associated with increased bleeding risk.[1-3] The precise influence of these factors is unclear and therefore guidelines are based on expert opinion and differ between countries.

The Dutch guideline (CBO) recommends to increase the trigger to $20x10^9$/L in case of fever and to $50x10^9$/L for the use of anticoagulation.[4] The British Committee for Standards in Haematology (BCSH), the American Society of Clinical Oncology (ASCO) and the American Association of Blood Banks (AABB) recommend likewise a more liberal transfusion policy in these conditions, but they don't specify triggers.[5-7] The same heterogeneity in recommendations is seen in case of invasive procedures, like lumbar punctures and insertion and removal of central venous catheters. So, the decision to transfuse is based on the opinion of the treating physician and may differ significantly. We performed an international survey to quantify these differences in order to establish in which situation the need for more knowledge is highest.

Material and methods

A survey was conducted among participants of the symposium of the foundation of Hemato-Oncologie voor Volwassenen Nederland (HOVON – the Haemato Oncology foundation for Adults in the Netherlands), and the congress of the European Hematology Association, (EHA), held in 2014.
Recorded data included characteristics of the respondents, determinants for alternative triggers, used triggers in specific situations and use of premedication. Regarding alternative triggers we asked about adherence to a prophylactic or therapeutic transfusion policy in autologous and allogeneic stem cell transplantation and which specific determinants they take into account when deciding to transfuse platelets to patients receiving myeloablative chemotherapy. Options were albumin, fibrinogen, liver function, renal function, C-reactive protein, fever, leukocyte count, platelet count, and hematocrit. In the questionnaire used at the EHA, bleeding and use of anticoagulants were added. Additionally, they were asked to specify which trigger they use in case of fever, severe mucosal damage, use of intravenous amphotericin B or asparaginase, bleeding in the previous five days,

use of different types of anticoagulants, insertion and removal of central venous catheter, and lumbar puncture. Besides platelets, they were asked if they give red blood cells to anemic thrombocytopenic patients to decrease the bleeding risk, and if yes, at which hematocrit.

Comparative statistics were used to describe the influence of experience on transfusion triggers. Seniors were defined as ≥11 year working as medical specialist or age ≥44 years, if work experience was not known.

Results and discussion

Respondents

Fifty-two hematologists filled in the questionnaire, 25 at the HOVON symposium and 27 at the EHA. All participated in the supportive care meeting, so they probably reflect a group of hematologists with special interest in transfusion medicine. Thirty respondents were Dutch, 15 came from nine other European countries and six from five countries outside Europe, one did not specify the country of origin. Median age of respondents was 43.5 years (range 30 to 70) with a median work experience as medical specialist of 11 years (range 0-36 years).

Risk factors

All respondents adhered to a prophylactic transfusion policy. For autologous stem cell transplantation 88% used a trigger of $10x10^9$/L and 12% of $20x10^9$/L. For allogeneic stem cell transplantation 83% used a trigger of $10x10^9$/L and 17% of $20x10^9$/L.

Platelet counts (98%), bleeding (97%) and use of anticoagulants (87%) were the most common determinants influencing transfusion decisions (Table 1). Years of experience did not influence the considered determinants, with exception of renal function which was more often taken into account by junior hematologists (30.4% versus 14.2%, 95%CI for difference -40.2 to 7.9). Thirty-eight percent of respondents additionally transfused erythrocytes to reduce the bleeding risk, using a mean hematocrit of 0.27% (range 0.21 to 0.40%). Rationale for this practice could be that erythrocytes are responsible for platelet margination to the vessel wall and the observed association between a higher hematocrit and delayed first bleed in acute myeloid leukaemia.[2]

Table 1. Determinants influencing the decision to transfuse

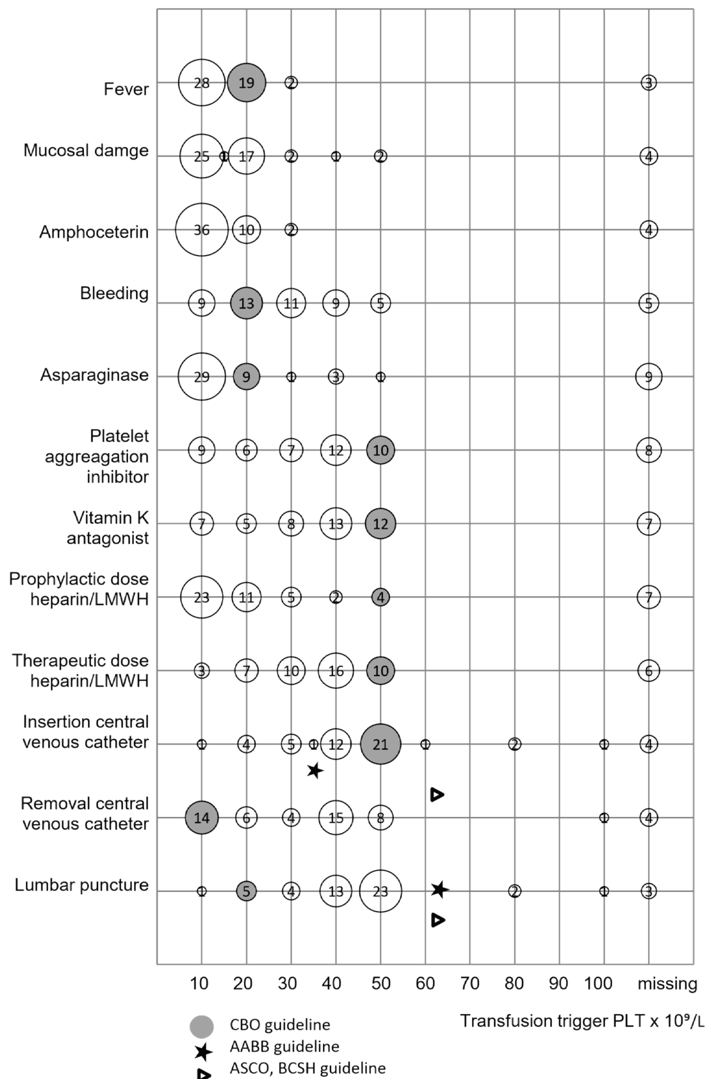
Parameter	Total n=52 (%)	Junior n=23 (%)	Senior n=21 (%)	Risk difference (%)	95% CI interval
Platelet count	51 (98.1)	22 (95.6)	21 (100)	4.4	-4.0; 12.6
Bleeding* (n=34)	33 (97.0)	15 (100)	16 (94.1)	5.9	-5.3; 17.1
Fever	32 (61.5)	14 (60.8)	14 (66.7)	5.8	-22.6; 34.1
Use of anticoagulants * (n=31)	27 (87.1)	14 (87.5)	12 (85.7)	1.8	-26.3; 22.7
Renal function	13 (25.0)	7 (30.4)	3 (14.2)	-16.1	-40.2; 7.9
Hematocrit	12 (23.1)	3 (13.0)	4 (19.0)	6.0	-15.7; 27.7
Fibrinogen	6 (11.5)	1 (4.3)	4 (19.0)	14.7	-4.0; 33.4
Leukocyte count	3 (5.8)	2 (8.7)	0 (0)	-8.7	-20.2; 2.8
CRP	2 (3.9)	2 (8.7)	0 (0)	-8.7	-20.2; 2.8
Liver function	2 (3.9)	1 (4.3)	1 (4.8)	0.4	-11.9; 12.8
Albumin	1 (1.9)	0 (0)	1 (4.8)	4.8	-4.3; 13.9
Other					
Need for invasive procedures	2 (3.9)	-	-	-	-
Splenomegaly	1 (1.9)	-	-	-	-
INR	1 (1.9)	-	-	-	-
ATG treatment	1 (1.9)	-	-	-	-

**Bleeding and use of anticoagulants were not standard items in the version used at the HOVON symposium, but mentioned in "others".*

Triggers

The triggers used in several situations are shown in figure 1. In case of fever, 54% of respondents used a trigger of $10x10^9/L$, whereas the Dutch guideline recommends a trigger of $20x10^9/L$. Although fever is associated with an increased risk of refractoriness[8], the influence on bleeding risk is less clear, which could explain this difference.[1,2] Before removal of a central venous catheter, 29% used a trigger of $10x10^9/L$, 12% of $20x10^9/L$ and 59% used triggers between 30 and $100x10^9/L$, whereas the guideline advises to maintain the trigger of $10x10^9/L$.[4] Before a lumbar puncture, 47% used a trigger of $40x10^9/L$ or lower, 47% a trigger of $50x10^9/L$ and 6% of at least $80x10^9/L$, although the recommended trigger is $20x10^9/L$.

Figure 1. Used transfusion triggers



The size of the bubbles and the numbers in the bubbles indicate the number of respondents. The recommended triggers by the CBO[4], ASCO[6], BCSH[7] and AABB[5] are marked. If no bubble is marked, the guideline does not specify a trigger for that situation.

Premedication

Six percent of respondents gave premedication to each patient, regardless of history. After a severe allergic reaction, 98% prescribed premedication for subsequent transfusions. Fifty-three percent gave antihistamines, which is in line with the guideline,[4] 10% gave corticosteroids, 29% a combination of these and 8% the combination and additionally paracetamol. According to a Cochrane review, routine administration of premedication is not effective in preventing allergic transfusion reactions.[9] In addition, paracetamol, diphenhydramine and a combination of both failed to reduce the incidence of allergic transfusion reactions in patients who previously experienced an allergic transfusion reaction.[10]

Conclusion

This study indicates large heterogeneity in transfusion policies. Guidelines mention risk factors for bleeding, but often refrain from recommending triggers and differ in recommendations before procedures. Although the majority of participants was Dutch and the survey was not validated, this study illustrates the need for evidence which trigger should be adhered in case of risk factors or invasive procedures.

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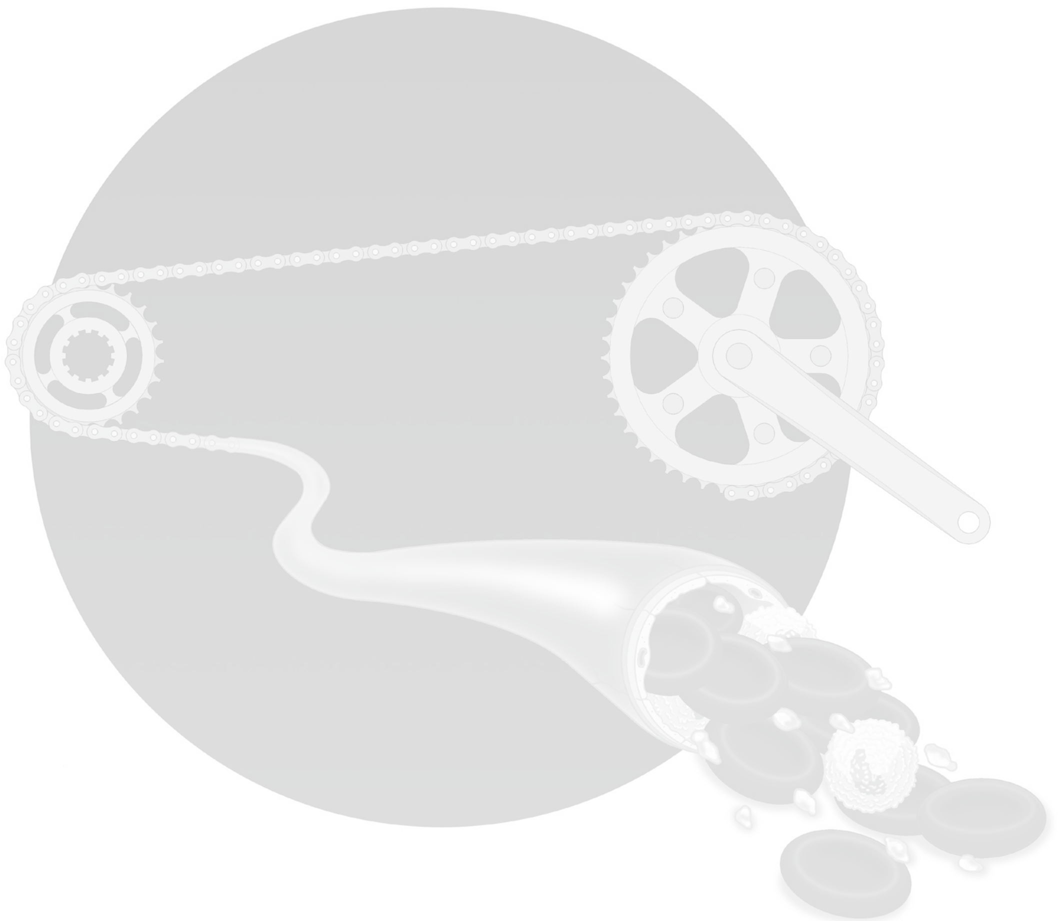

Chapter 3

Efficacy and availability of HLA-matched platelet transfusions for refractory patients in the Netherlands

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Abstract

Background

Refractory patients who have an one hour corrected count increment (1h CCI) ≤7.5 on two transfusions with random platelet concentrates may benefit from HLA matched transfusions. We aimed to evaluate our HLA matched donor program and to quantify the increases in platelet counts after HLA matched platelet transfusions.

Methods

A cohort study was performed among patients who received an HLA matched platelet concentrate in the Netherlands between 1994 and 2017. Per patient the number of available split matched donors was determined. For patients with five or less donors, the ethnic background was assessed using HaploStats. We selected the first transfusion at which a patient was exposed to a new antigen. One hour corrected count increments (1hCCI) after mismatched transfusions were compared with matched transfusions using mixed model linear regression, adjusted for within patient variation. In addition, the effect of AB0 mismatches was investigated. Subgroup analyses were performed for patients with positive and negative antibody screen.

Results

A total of 1,206 patients received 12,350 HLA matched transfusions. In September 2017, 19,478 donors were HLA typed. Median 83 (interquartile range 18; 266) donors were available per patient. For 95 (10.3%) of patients five or less donors were available. The 1h CCI after a matched transfusion was 14.09 (95% reference interval 1.13; 29.89). This decreased with -1.94 (95% confidence interval (CI) -3.15; -0.74) after an HLA mismatched transfusion and with -3.70 (CI -5.22; -2.18) after a major AB0 mismatched transfusion. In patients with negative alloantibody screening tests, mismatches did not influence the 1h CCI.

Conclusion

HLA matched platelet support could be offered for the majority of refractory patients. Matched platelet concentrates yielded the highest 1h CCI, whereas mismatched transfusions still result in adequate increments.

Introduction

Repeated insufficient count increments after platelet transfusions, i.e. refractoriness, is a common problem in patients who regularly need platelet transfusions. Patients are refractory when the one hour corrected count increment (1h CCI) is ≤7.5 after two subsequent ABO compatible platelet transfusions.[1] Refractoriness is associated with an increased risk of bleeding, prolonged hospital stay, and higher hospital costs.[2,3] In 80-90% of platelet refractory patients, this is caused by non-immune mediated factors like fever, infection, splenomegaly, bleeding, and use of medications. Immune mediated clearance of transfused platelets is predominantly caused by alloantibodies directed against human leukocyte antigen (HLA) class I A or B antigens.[4,5] If these antibodies are directed against a high-frequency HLA class I antigen, it is likely that these are incompatible with the platelets of at least one of the five donors of a random platelet concentrate resulting in rapid clearance of the transfused platelets.[4] Less commonly, antibodies directed against HPA, human platelet antigens, or a high titer of AB0 alloantibodies induce the accelerated destruction of transfused platelets.[6-9]

The pathophysiology of HLA-alloantibody development has not been elucidated completely. Known risk factors are pregnancy and transfusion of non-leukoreduced blood products. Against the odds, there seems to be no clear dose-response relationship with the number of transfused platelet concentrates, and not all immunized patients exhibit refractoriness for platelet transfusions.[3,9-12]

Refractory patients may benefit from AB0 identical or compatible, HLA class I matched platelet transfusions, especially with respect to the 1h CCI. Studies are less consistent regarding the effect of matching on 24h CCI.[4,10] Matching is performed on HLA class I A and B antigens. Platelets do also express HLA class I C antigens, but this expression is lower and clinically relevant antibodies are hardly formed.[2,3] In the Netherlands, a large panel of donors has been HLA-typed and they can be requested to donate platelets for specific HLA-matched, refractory patients.

In the current study, we aimed to evaluate our HLA-matched donor program by estimating the proportion of the Dutch patient population that can be supported by the current HLA-typed donor population. Additionally, we aimed to compare the effect of different matching strategies on the efficacy of platelet transfusions expressed as 1h CCI in refractory patients.

Methods

Design and population

We performed a cohort study using a registry of clinically refractory patients for whom an HLA matched product was ordered at the Unit for Transfusion Medicine of Sanquin, the Dutch blood supply organization. This registry started in 1994 and has nationwide coverage since 2013. An HLA-matched product can be requested for patients with inadequate increments (1h CCI ≤7.5) on at least two platelet transfusions and for which a role of HLA antibodies is suspected, regardless whether HLA antibodies have been detected yet. All HLA-matched products are derived via apheresis, leukoreduced, stored in plasma, and irradiated.[1]

Since the start of the registry, HLA typing techniques have improved significantly, and nowadays DNA-based typing has replaced serological typing. In the current study, we only included patients and donors who had been HLA typed at a split antigen level. Split antigens can be distinguished from other antigens in the same broad group by the presence of unique or private epitopes.[13] Neonates were excluded from all analyses, since thrombocytopenia of immunological origin in neonates is predominantly caused by transferred antibodies from the mother.[14,15] Although some patients may have had HPA antibodies and received a product also matched on HPA, we did not take matching on HPA into account for the current study, as not all donors and patients are HPA typed.

Available donors

For all patient phenotypes in the registry, we determined the number of available split antigen matched donors in the current HLA typed platelet donor population, regardless of AB0 blood group. A donor-to-patient match was categorized as split matched if all donor HLA A and B antigens were present in the patient's genotype, i.e. patient and donor were HLA identical or compatible. We assumed that the distribution of the phenotypes of patients in the registry is representative for the phenotypes in the Dutch patient population. We used HaploStats to estimate the most likely population of origin. HaploStats is an algorithm from the National Marrow Donor Program, which estimates the most likely phased genotype based on data of a large US reference population. Subsequently, the prevalence of the haplotype is given among African-Americans, Asian or Pacific Islanders, Caucasians, Hispanics, and Native Americans.[16,17] The ethnicity with the highest prevalence for a certain genotype was determined as most likely for that patient. For patients with five or less donors we compared the most likely ethnicity with that of a random

sample of 100 patients with at least 30 donors. Next, we assessed the prevalence of A and B antigens among patients and donors.

Matching and CCI

We compared the 1h CCI after a matched transfusion with the 1h CCI after a mismatched transfusion. A donor-to-patient match was categorized as mismatch when the donor HLA type contained an antigen that was not present in the patient. Mismatched antigens could be selected based on either the HLA antibody specificity, epitope matching, CREGs, the effect of previous transfusions, or any combination of these strategies.
For this analysis, we established two cohorts: the 'new antigen cohort' and the 'new donor cohort'. In the new antigen cohort, only selected the first matched transfusion and the first transfusion at which a patient was exposed to a new antigen was selected in order to prevent bias which could arise by knowledge about patient-specific responses to previous mismatched transfusions with the same HLA phenotype. In the new donor cohort, the first transfusion of a donor per patient was selected in order to investigate additional donor-specific effects. The identity of the donor was optionally recorded since 2006 and this was universally done since 2008. Exclusively patients for whom the identity of the donor of all transfusions could be retrieved were included in the new donor cohort. In both cohorts, several transfusions per patients could be included. To adjust for within patient correlations, we used a mixed model linear regression with a random intercept per patient.

We performed four additional analyses. First, we explored the effect of AB0 blood group incompatibility, as high levels of anti-A and anti-B could result in refractoriness.[11,18] In our country, AB0 blood group compatibility is taken into consideration only if feasible given the number of HLA matched donors. Minor AB0 incompatibility was defined as the presence of anti-A and/or anti-B alloantibodies in the product directed against patient's blood group antigens and major AB0 incompatibility as the presence of anti-A or anti-B alloantibodies in patient plasma directed against donor blood group antigens.[19] The 1h CCI after minor or major AB0 incompatible transfusions was compared with AB0 identical transfusions, conditional on HLA matching.

Second, we quantified the effect of the first matched transfusion. It could be hypothesized that the increment of the first HLA matched transfusion is relatively low, because the patient has been thrombocytopenic for a longer period of time and

thus platelets may disappear rapidly from the circulation to restore vascular integrity. We compared the 1h CCI of the first matched transfusion with the 1h CCI of subsequent matched transfusions. This analysis was performed only in the new donor cohort.

Third, we examined whether the effect of matching differs among patients with or without the presence of HLA antibodies. The results of the HLA antibody testing are not necessarily available at the moment the first HLA matched transfusions are ordered and some patients remain negative in all antibody tests. Subgroups were defined based on the results of the HLA antibody screening test as performed by Luminex or Complement Dependent Cytotoxicity (CDC) tests.

Fourth, we investigated whether determination of the specificity of HLA antibodies with the Luminex Single Antigen (LSA) test improves the quality of matching. Mismatches were categorized as acceptable when the patient had no antibodies directed against the mismatched antigens. In this analysis, atients for whom the HLA antibody specificity had not been tested were excluded.

Results

Between 1994 and 2017, an HLA matched platelet concentrate was requested for 1,206 refractory patients, of which 1,021 were typed at antigen split level (figure 1). These patients received in total 12,350 HLA-matched transfusions, with a median of 5 (interquartile range (IQR) 2; 15) transfusions per patient and a maximum of 229 transfusions per patient. Patients were on average 54.4 years old, 65.4% was female and the majority suffered from a malignant hematological disease, predominantly acute leukemia (table 1).

Table 1. Demographics of the study population.

Characteristics	Total registry	New antigen cohort	Split matched transfusion	Mismatched transfusion
Patients (n)	1,021	581	427	290
Female gender (%)	666 (65.4)	385 (66.3)	285 (66.7)	194 (66.9)
Age at first transfusion, mean (SD)	54.4 (16.3)	54.1 (16.2)	55.2 (15.1)	51.8 (17.6)
Diagnosis				
Acute leukemia	404 (39.6)	278 (47.9)	214 (50.1)	137 (47.2)
Chronic leukemia	49 (4.8)	29 (5.0)	25 (5.9)	13 (4.5)
Lymphoma	51 (5.0)	26 (4.5)	17 (4.0)	12 (4.1)
Multiple myeloma	29 (2.8)	16 (2.8)	7 (1.6)	9 (3.1)
Myelodysplastic syndrome	123 (12.1)	47 (8.1)	36 (8.4)	22 (7.6)
Myelofibrosis or aplastic anemia	81 (7.9)	50 (8.6)	37 (8.7)	33 (11.4)
Benign hematological diseases*	23 (2.3)	12 (2.1)	6 (1.4)	10 (3.5)
Solid tumor	27 (2.6)	14 (2.4)	11 (2.6)	4 (1.4)
Solid organ transplantation	7 (0.7)	5 (0.9)	2 (0.5)	4 (1.4)
Other or unknown	166 (16.3)	104 (17.9)	72 (16.9)	46 (15.9)
Number of transfusions per patient, median (IQR)	5 (2; 15)	1 (1; 2)	1 (1; 1)	1 (1; 3)
Number of transfusions	12,350	1,068	427	641
Year of transfusion				
1994-2000 (%)	817 (6.6)	113 (10.6)	36 (8.4)	77 (12.0)
2001-2006 (%)	1,961 (15.9)	210 (19.7)	68 (15.9)	142 (22.2)
2007-2011 (%)	2,371)19.2)	263 (24.6)	109 (25.5)	154 (24.0)
2012-2017 (%)	7,201 (58.3)	482 (45.1)	214 (50.1)	268 (41.8)

*The total registry contains all patients in the registry. The new antigen cohort contains the first transfusion at which a patient was exposed to a new antigen. * Including Glanzmann thrombasthenia, Bernard Soulier syndrome, Castelman's disease, grey platelet syndrome, thalassemia, polycythemia vera, auto-immune thrombocytopenia, immune thrombocytopenia.*

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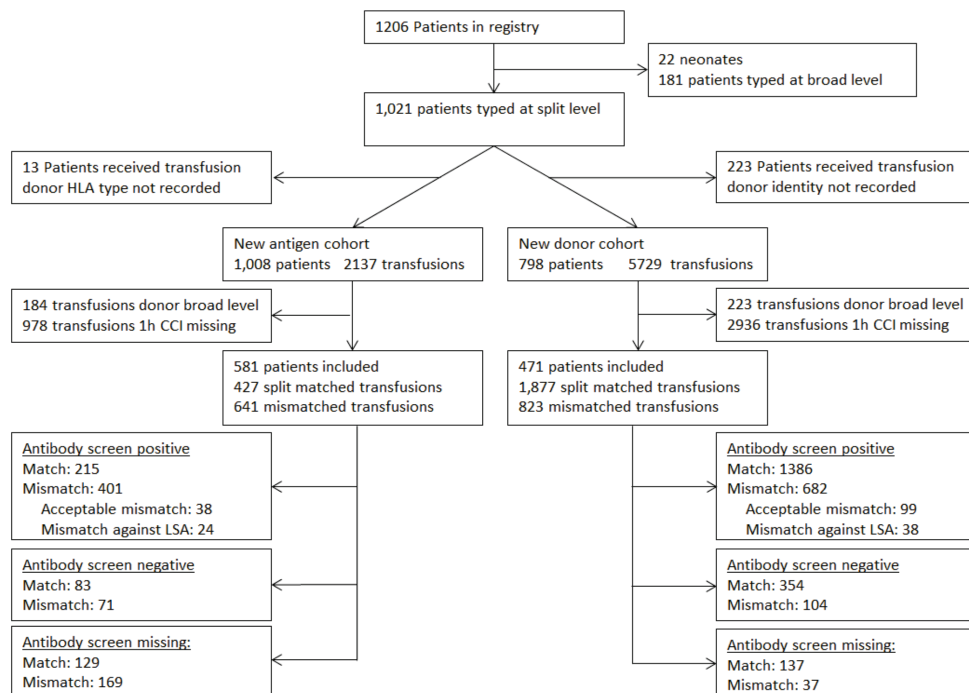
Figure 1. Flow chart of data handling

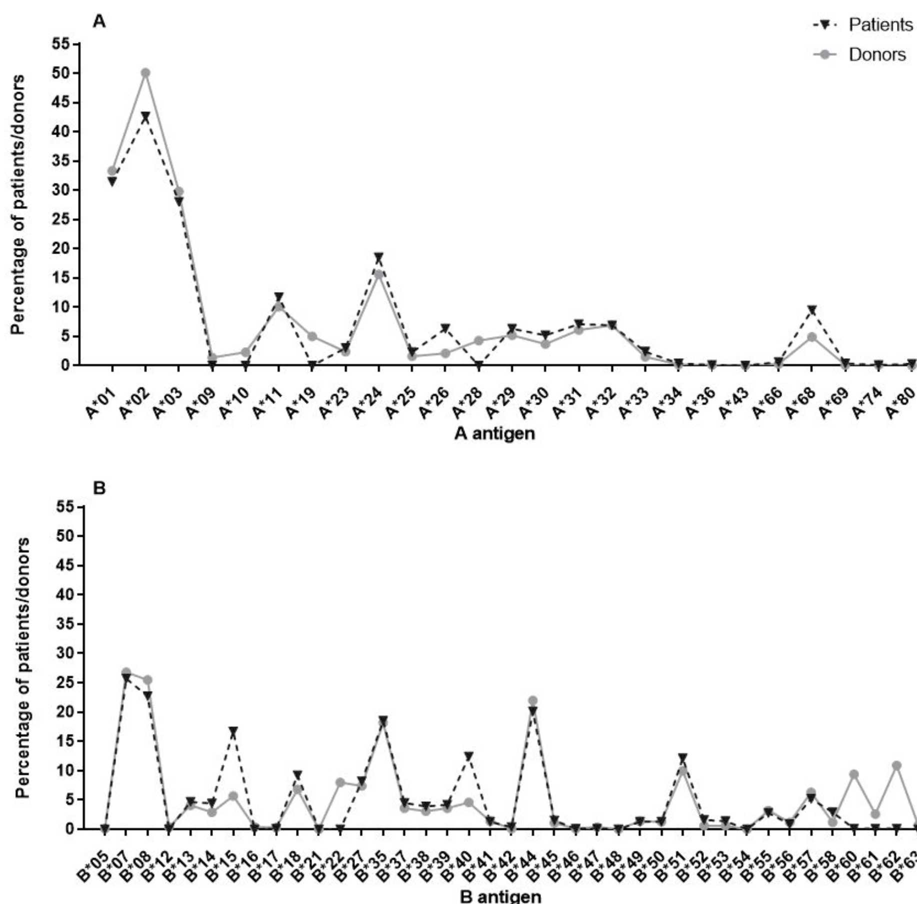


Available donors

In September 2017, the registry contained 19,478 HLA typed platelet donors, of whom 16.132 donors had been typed at a split antigen level. They had 4,770 unique phenotypes, with a median of 8 (IQR 2; 30) donors per phenotype. The prevalence of HLA A and B antigens was comparable between patients and donors (figure 2). The most common phenotype among the donors was homozygous *HLA A*01; B*08*, which was expressed by 251 donors. The 1,021 patients for whom HLA typed platelets had been requested expressed 701 different HLA phenotypes, with a median of 1 (IQR 1; 2) patient per phenotype. The most common phenotype, expressed by 16 patients, was *HLA A*01, A*02; B*07,B*08*, for which 193 identical and 582 compatible donors were available. Each patient could be matched to a median of 83 (IQR 18-266) identical or compatible donors, with a maximum of 807 donors per patient (figure 3). For 17 patients, all with a unique phenotype, no matched donor was available. For 95 (9.3%) patients five or less matched donors could be found, for 161 (15.7%) patients ten or less, and for 251 (24.6%) patients twenty or less donors were registered. For patients with ≤5 donors, the most likely populations of origin were Caucasian (28 patients, 29%) Asian-Pacific (23 patients,

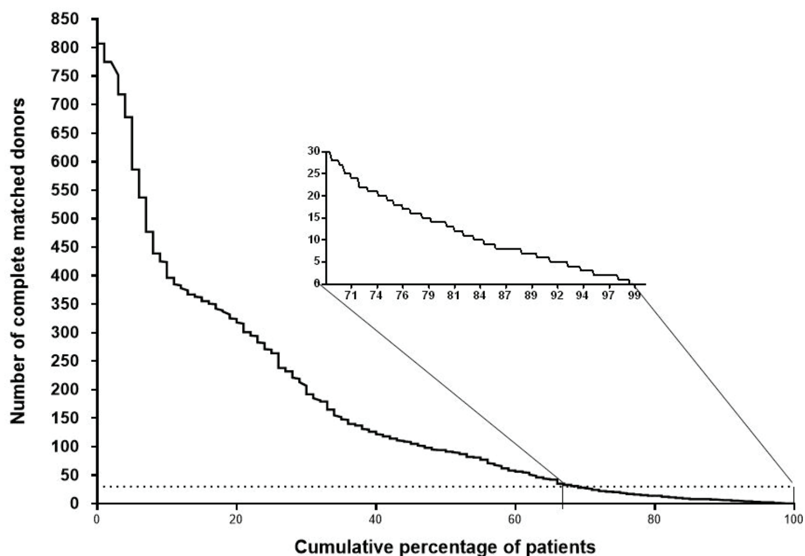
24%), and African-American (20 patients, 21%). For a random sample of 100 patients with at least 30 donors, (median 155 donors, IQR 86 to 365), this was Caucasian (74%), Native American (15%), and Hispanic (6%) (figure 4).

Figure 2. Prevalence of antigens among split typed patients and donors.



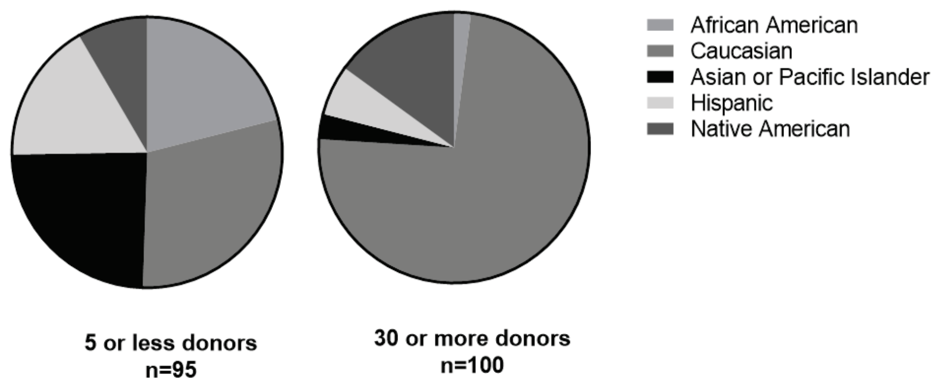
The percentage of donors with certain antigen is depicted with circles and the grey solid line. The prevalence among patients is reflected with triangles and a black dashed line. Panel A: HLA-A antigens Panel B: HLA-B antigens

Figure 3. Number of complete matched donors in the current donor population for all patients in the registry



The number of HLA identical or compatible donors per patient. The dotted line is set at 20 donors.

Figure 4. Most likely population of origin for patients with ≤5 donors (A) and for patients with ≥30 donors (B)



Level of matching

The new antigen cohort comprised 1,068 transfusions issued to 581 patients, with median 1 (IQR 1; 2) transfusion per patient (table 1). Forty percent of the transfusions were matched and the remaining transfusions were mismatches which introduced a new antigen. The average 1h CCI following a matched transfusion was 14.09 (95% reference interval (RI) 1.13 to 29.89). A mismatched transfusion was associated with a reduction of the 1h CCI of 1.94 (95% confidence interval (CI) -3.15 to -0.74) (table 2). Major AB0 incompatibility was associated with a reduction of the 1h CCI of 3.70 (CI -5.22 to -2.18), and this reduction was 1.06 (CI -2.65 to 0.52) for minor AB0 incompatibility.

The new donor cohort consisted of 2,700 transfusions, 471 patients and 1,698 unique donors (figure 1). Patients received a median of 8 (IQR 2; 21) transfusions from different donors and donors donated platelets for a median of 1 (IQR 1; 2) patient with a maximum of 13 patients per donor. Demographic characteristics of this selected cohort are depicted in table 1 of the supplemental material. The mean 1h CCI after a matched transfusion was 14.7 (RI 1.89 to 29.56). A mismatch was associated with a reduction of the 1h CCI of 1.59 (CI -2.47 to -0.71) (table 2). Major AB0 incompatibility was associated with a reduction of the 1h CCI of 3.02 (CI -3.87 to -2.17), and this was -0.79 (CI -1.69 to 0.11) for minor AB0 incompatibility. As compared to the first matched transfusion, subsequent matched transfusions are associated with a reduction of 1h CCI of -0.41 (CI -1.23 to 0.41).

The HLA alloantibody screen was positive for 295 patients in the new antigen cohort and for 305 patients in the new donor cohort. In patients with a positive screening result, the CCI was -3.09 (CI -4.68 to -1.50) lower in the new antigen cohort after a mismatched transfusion, and this was -1.86 (CI -2.89 to -0.84) in the new donor cohort. Results of the antigen specific test were available for 62 transfusions in the new antigen cohort and 137 transfusions in the new donor cohort (figure 1). A mismatch with acceptable antigens according to the antigen specific test was not significantly associated with the 1h CCI in both cohorts. A mismatch with an antigen against which the patient had antibodies was associated with a reduction of the 1h CCI with -3.98 (CI -7.14 to -0.82) in the new donor cohort. This was -2.19 (CI -4.58 to 0.19) in the new antigen cohort (table 3). In patients with a negative antibody screen, a mismatched transfusion was not associated with the 1h CCI in either of the two cohorts (table 2).

Table 2. Corrected count increments according to different matching strategies.

Matching	Number (%)	Difference 1h CCI* (95% CI)	Adjusted AB0 incompatibility (95% CI)
New antigen cohort			
Matched	*427 (40.0)*	*Ref*	*Ref*
Mismatch	641 (60.0)	-1.94 (-3.15; -0.74)	-1.99 (-3.28; -0.71)
Minor AB0 incompatibility	187 (23.3)	N/A†	-1.06 (-2.65; 0.52)
Major AB0 incompatibility	177 (22.0)	N/A†	-3.70 (-5.22; -2.18)
Patients with positive alloantibody screen			
Matched	215 (34.9)	*Ref*	*Ref*
Mismatch	401 (65.1)	-3.09 (-4.68; -1.50)	-3.28 (-4.97; -1.59)
Minor AB0 incompatibility	125	N/A†	-1.55 (-3.60; 0.49)
Major AB0 incompatibility	119	N/A†	-4.00 (-5.93; -2.09)
Patients with negative antibody screen			
Matched	83 (53.9)	*Ref*	*Ref*
Mismatch	71 (46.1)	-0.26 (-2.75; 2.21)	0.28 (-2.14; 2.71)
Minor AB0 incompatibility	24 (19.1)	N/A†	-0.27 (-3.48; 2.92)
Major AB0 incompatibility	27 (24.4)	N/A†	-2.87 (-5.82; 0.08)
New donor cohort			
Matched	*1,877 (69.5)*	*Ref*	*Ref*
Mismatch	823 (30.5)	-1.59 (-2.47; -0.71)	-1.32 (-2.21; -0.43)
Minor AB0 incompatibility	513 (20.5)	N/A†	-0.79 (-1.69; 0.11)
Major AB0 incompatibility	414 (16.6)	N/A†	-3.02 (-3.87; -2.17)
Patients with positive alloantibody screen			
Matched	1,386 (67.0)	*Ref*	*Ref*
Mismatch	682 (33.0)	-1.86 (-2.89; -0.84)	-1.64 (-2.66; -0.61)
Minor AB0 incompatibility	392 (20.2)	N/A†	-0.49 (-1.54; 0.56)
Major AB0 incompatibility	313 (16.1)	N/A†	-3.12 (-4.11; -2.14)
Patients with negative antibody screen			
Matched	354 (77.3)	*Ref*	*Ref*
Mismatch	104 (22.7)	-0.47 (-2.36; 1.41)	0.18 (-1.76; 2.12)
Minor AB0 incompatibility	88 (21.8)	N/A†	-2.62 (-4.53; -0.72)
Major AB0 incompatibility	66 (16.3)	N/A†	-3.65 (-5.57; -1.72)

*Results are shown for the new antigen and new donor cohort and stratified on result of the antibody screening. Patients for whom no antibody screening was performed or results were missing, were excluded from the stratified analyses. *CCI corrected count increment. †N/A not applicable in the crude analysis*

Table 3. The effect of matching on 1h CCI in patients with alloantibodies related to the results of the Luminex Single Antigen test (LSA)

	New antigen cohort		New donor cohort	
Matching	**Number (%)**	**Difference 1h CCI* (95% CI)**	**Number (%)**	**Difference 1h CCI* (95% CI)**
Split matched	*215 (77.6)*	*Ref*	*1,386 (91.0)*	*Ref*
Acceptable mismatch	38 (13.7)	-3.08 (-6.32; 0.15)	99 (6.5)	-2.19 (-4.58; 0.19)
Mismatch against LSA	24 (8.7)	-3.11 (-6.94; 0.73)	38 (2.5)	-3.98 (-7.14; -0.82)

**CCI corrected count increment*

Discussion

In the Netherlands, currently, almost 20,000 donors, representing 6,717 unique HLA phenotypes, are available to donate HLA matched platelets for refractory patients upon request. The prevalence of HLA A and B antigens among these donors largely overlaps with the prevalence of these antigens among patients. Still, for 10.3% of refractory patients no sufficient number of completely matched donors were available, assuming that at least five donors are required to ensure sufficient support during intensive treatment for leukemia, which is the most common condition among refractory patients. A completely matched donor is preferred, as this yielded statistically significant higher corrected count increments. However, mismatched transfusions, the only alternative if completely matched donors are unavailable, still lead to adequate corrected count increments. The 1h CCI after a mismatched transfusion was approximately 12, whereas the 1h CCI after random platelet concentrates was ≤7.5 for these patients, which is the cut off to request HLA matched transfusions for refractory patients.[1,3] In comparison, the average 1h CCI after plasma stored random platelet concentrates was 17.1, as has been shown by a previous Dutch trial.[20]

To the best of our knowledge, this study is the first to evaluate the current practice for platelet refractory patients in a large population of patients and donors. A strength of this study is that we were able to adjust for within patient correlations, as patient characteristics largely influence the effect of platelet transfusions. In addition, we selected only the first transfusion at which a patient was exposed to a new foreign antigen, because the effect of the previous mismatched transfusion could influence the selection for the next transfusion. Limitation of the study is that results of the HLA antibody specificity testing were only available for a minority of

patients, limiting the power of this analysis. This is partly explained by the large time span of the registry and the relative recent introduction of the Luminex Single Antigen test. Moreover, results for the test could not be retrieved from all hospitals. However, we do not expect selection bias due to missing data as most patients suffered from a hematological malignancy and the accompanying treatment is highly protocolled.

All antigens present in the patients´ phenotypes are represented in the donor population, as we showed by counting the antigens. However, for a relevant proportion of refractory patients were not sufficient donors available, which indicates that counting antigens is not an adequate method to evaluate the donor program. The HLA system is the most polymorphic part of the human genome and many combinations of antigens are possible with haplotype-specific linkage disequilibrium patterns.[7,21] Therefore, an adequate evaluation needs to be done on a haplotype or phenotype level.

Approximately 10% of donors are no longer callable and need to be replaced each year. New donors preferably increase the variability of the donor population by adding new phenotypes. For organ or stem cell transplantation, it has been shown that for patients from ethnic minorities the probability to find an HLA compatible donor is lower as compared to Caucasian patients.[22,23] We hypothesized that HLA matched platelet support is hampered in a similar way. We estimated the most likely population of origin based on HLA phenotype, as recording of patient and donor ethnicity is prohibited in the Netherlands. Therefore,. The validity of this algorithm is high, but some misclassification cannot be ruled out.[24] A relatively large proportion of patients for whom less than five donors were available, had a non-Caucasian background, predominantly Asian Pacific Islander and African American. The preponderance of non-Caucasian patients in the group with insufficient donors suggests that additional typing and recruitment among ethnic minorities could increase the likelihood to find a compatible donor, as it would increase the variety of phenotypes among the donor population. The benefits of additional typing of ethnic minorities would differ per population of origin. It would be especially suitable for patients with an Asian background, as the genetic variability in this population is low. The homogeneity is so large that Japan, for example, irradiates all blood products to prevent transfusion associated graft-versus-host disease.[25] In contrast, finding a matched donor will remain difficult for patients with an African American background, as the genetic variability is known to be significantly higher as compared to Caucasians and Asians.[22] Moreover, due to the highly polymorphic

feature of the HLA system disparities between patients and donors will sustain, especially with increasing admixture in the population.[26]

Transfusion of a completely HLA matched donor yielded the highest corrected count increments in both established cohorts. When matched donors are not available, acceptable antigens can be determined by HLA antibody specificity testing. In patients with alloantibodies, a mismatch with acceptable antigens seems preferable, but is still associated with a marked reduction in CCI. However, this effect was not statistically significant and the sample size for this analysis was small. In patients with negative alloantibody screening, matching on HLA did not improve the increments. So, our findings support the recommendation to treat these patients with random platelet concentrates.[3,5]

In all patients, major AB0 mismatches significantly reduced the CCI, suggesting that AB0 identical platelets should be pursued, especially when no HLA matched donor is available. This is in line with the results of a systematic review of 19 studies among hematological and oncological patients which showed consistently higher increments for AB0 identical platelet transfusions.[18]

In conclusion, refractory patients with positive alloantibody tests benefit considerably from HLA matched, AB0 identical platelet transfusions, but mismatched transfusions also improve platelet counts. In patients with a negative alloantibody test, HLA matched transfusions have no additional beneficial effect as compared to mismatched transfusions on the 1h CCI. Although a large donor population is available for donation of HLA matched platelets, adequate transfusion support could not be guaranteed for all refractory patients. Additional recruitment among non-Caucasian subjects might increase the availability of matched donors for all refractory patients.

Supplementary material

Supplementary material is available from the author upon request.

Table S1. Demographics of the new donor cohort

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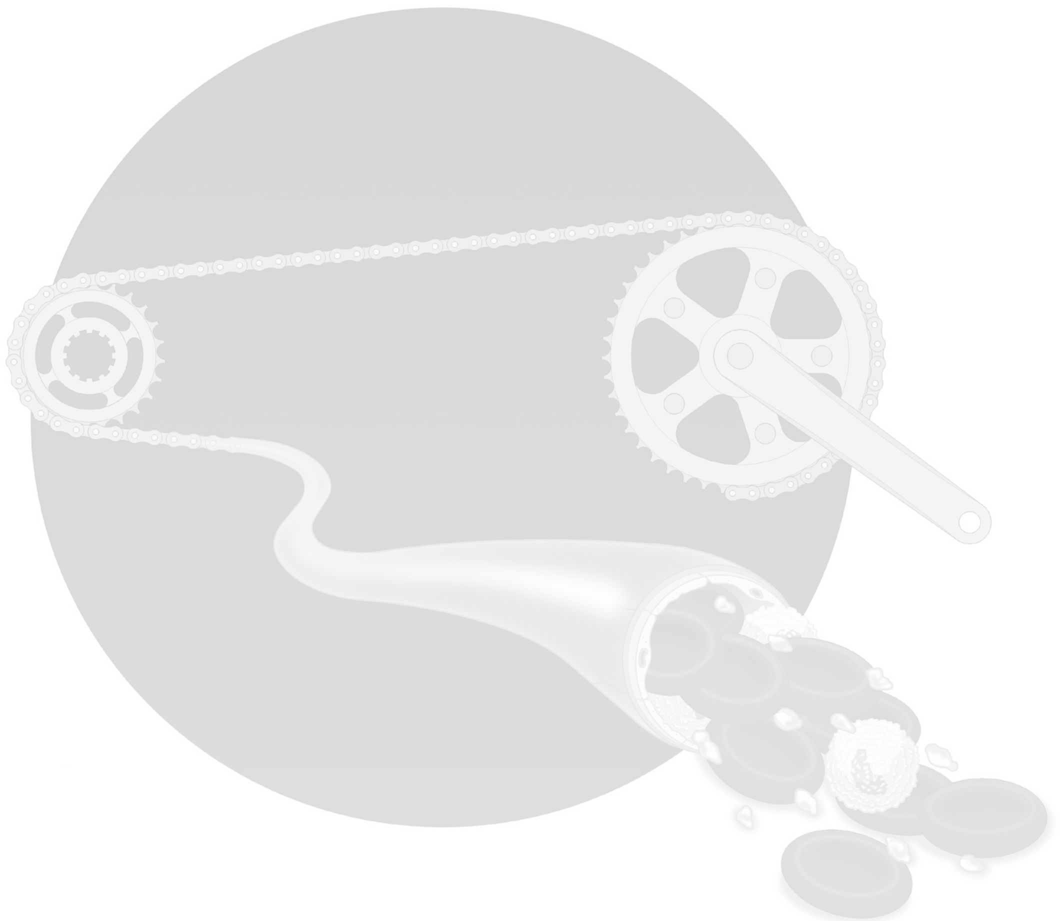

Chapter 4

The identification of cases of major hemorrhage during hospitalization in patients with acute leukemia using routinely recorded healthcare data

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Abstract

Introduction

Electronic health care data offers the opportunity to study rare events, although detecting these events in large datasets remains difficult. We aimed to develop a model to identify leukemia patients with major hemorrhages within routinely recorded health records.

Methods

The model was developed using routinely recorded health records of a cohort of leukemia patients admitted to an academic hospital in the Netherlands between June 2011 and December 2015. Major hemorrhage was assessed by chart review. The model comprised CT-brain, hemoglobin drop, and transfusion need within 24 hours for which the best discriminating cut off values were taken. External validation was performed within a cohort of two other academic hospitals.

Results

The derivation cohort consisted of 255 patients, 10,638 hospitalization days, of which chart review was performed for 353 days. The incidence of major hemorrhage was 0.22 per 100 days in hospital. The model consisted of CT-brain (yes/no), hemoglobin drop of ≥0.8 g/dl and transfusion of ≥6 units. The C-statistic was 0.988 (CI 0.981-0.995). In the external validation cohort of 436 patients (19,188 days), the incidence of major hemorrhage was 0.46 per 100 hospitalization days and the C-statistic was 0.975 (CI 0.970-0.980). Presence of at least one indicator had a sensitivity of 100% (CI 95.8-100) and a specificity of 90.7% (CI 90.2-91.1). The number of days to screen to find one case decreased from 217.4 to 23.6.

Interpretation

A model based on information on CT-brain, hemoglobin drop and need of transfusions can accurately identify cases of major hemorrhage within routinely recorded health records.

Introduction

Electronic health care data are increasingly used for research purposes.[1-3] It offers the potential to investigate rare events and to obtain reliable estimates using large populations or specific subgroups with long follow-up time, while maintaining high external validity.[3-5]

Within the field of hematology, studies regarding bleeding could benefit from electronic health care data. Bleeding can be categorized according to the WHO criteria, a scale from 1 to 4, in which grade 1 indicates petechiae and grade 4 debilitating blood loss.[6] Major hemorrhages (WHO grade 3-4) are clinically most relevant, but occur infrequently. To obtain sufficient power, many studies use a composite endpoint consisting of all bleeding events WHO grade ≥2.[7-10] However, it has been suggested that including WHO grade 2 bleedings in a composite outcome is not valid.[11] Instead, it would be preferable to include only hemorrhages WHO grade 3 and 4, although this would require large sample sizes.

Several algorithms have been developed to identify bleeding events from administrative data and these are mostly based on billing data or ICD codes.[12] The reliability of such an algorithm depends upon the quality of the administrative coding and regional and temporal variation exists.[13] In contrast to billing data and ICD codes, routinely recorded clinical data, like laboratory measurements, are more objective and could therefore potentially be used to improve the identification of bleeding events.[12] These data are easily obtainable and do not require any additional effort by clinicians. The aim of this study was to develop a model to identify patients with a high likelihood of major hemorrhage (WHO grade 3-4) within a database of routinely recorded clinical data of adult patients with acute leukemia without a detailed review of patient files.

Methods

Setting and population

The model was developed using routinely recorded clinical data of a cohort of adult patients with acute leukemia admitted to the Leiden University Medical Center in the Netherlands between June 2011 and December 2015. The model was externally validated within a cohort of adult acute leukemia patients admitted to the University Medical Center Utrecht or to the Maastricht University Medical Center between January 2010 and January 2016.

In all cohorts, patients were selected based on the 'diagnosis treatment combination' code (in Dutch 'DBC, diagnose behandel combinatie'). The DBC code is a national system for the registration and reimbursement of health care activities.[14]. Patients with acute lymphatic or myeloid leukemia, or refractory anemia with excess blasts (RAEB) were included in this study (DBC codes 756, 761, and 762). The study protocol was approved by the Medical Ethical Committee of the Leiden University Medical Hospital, University Medical Center Utrecht, and Maastricht University Medical Center, and the scientific committee of the Center for Clinical Transfusion Research, Sanquin. All data were pseudonymized and the ethical committees waived the requirement for informed consent.

<u>Variables</u>

Routinely recorded clinical data were extracted from the electronic health care system of the hospitals. Collected variables were age, gender, DBC codes, dates of hospitalizations, received blood products, hemoglobin measurements, and dates of CT-scans of the brain. Drop in hemoglobin per 24 hours was categorized into ≤0.8, >0.8 up to and including 1.6g/dl, >1.6 to 1.9 g/dl, >1.9 to 2.2 g/dl, >2.2 to 2.8 g/dl and >2.8 g/dl. Transfusion need was defined as total number of blood products per 24 hours, including red blood cells, platelets and plasma and categorized in ≤2, 3, 4, 5, and ≥6 blood products.

Information about bleeding was collected via chart review and classified according to the WHO Severity Grading System with the specifications as used in the PlaDo trial: grade 1 petechiae, grade 2 mild blood loss, grade 3 gross blood loss, grade 4 debilitating blood loss (online supplements, table S1).[6,15] Major hemorrhage, WHO grade 3 or 4, was taken as primary outcome. Secondary, all bleedings, regardless of WHO grade, were included.

<u>Sample</u>

Chart review was performed for a sample of observation days during hospital admission, selected according to the following strategy. All eligible hospitalization days were first stratified by categories of hemoglobin drop and number of transfusions, and from each of these strata we aimed to include 20 days. Additionally, all days on which a CT-brain was performed were reviewed. To ensure no bleeding was missed due to patient or doctor's delay, a time frame of one day before and one day after the selected date was reviewed. As a negative control, we selected 90 days on which maximal one blood product was transfused and the drop in hemoglobin was less than 0.8 g/dL. Sampling was performed without replacement

and restricted to one day per hospital admission per indicator. Using this selection procedure, the sample was enriched with days with a potentially increased risk of bleeding. To adjust for this, the sample was weighted according to the prevalence of the indicators in the original cohort for all analyses and the calculation of the incidence of hemorrhage. With the final sample of 352 hospitalization days, we could establish a specificity of 96% with a precision of 2% and an alpha of 0.05, assuming an incidence of 0.5 cases per 100 hospitalization days.

Development of the model

The results of the chart review were used as golden standard for the outcome of major hemorrhage. Drop in hemoglobin per 24 hours and transfusion need per 24 hours were taken as indicators for major blood loss and CT-brain during hospital stay as an indicator for potential intracranial hemorrhage. A logistic model was fitted to predict the risk of major hemorrhage. For all indicators the sensitivity, specificity, negative and positive predictive value, and C-statistic were calculated. For the continuous predictors, the cut-off value with the best discriminative capacity was entered into the model. Discrimination is the ability to separate patients who had a hemorrhage from those who had not and is quantified by the C-statistic. A C-statistic of 1.0 denotes perfect discrimination and a C-statistic of 0.5 represents discrimination equivalent to random chance.[16] The model was internally validated using bootstrap resampling with 100 repetitions. Performance of the model was expressed by the sensitivity, specificity, negative and positive predictive value with exact binomial 95% confidence intervals and summarized by the C-statistic. In addition, we calculated the number of days needed to screen to detect one case of major hemorrhage for all predicted risks.

External validation

The model was externally validated in a cohort of leukemia patients from two other academic hospitals in the Netherlands. The same methods as in the derivation cohort were used to select the patients and extract the required data. The predicted risk of major hemorrhage was calculated using the model. Chart review was performed for all days with a predicted risk >0.01, 100 random control days with a predicted risk of 0.006, and 100 control days with a predicted risk of 0.0002. Discriminative capacity was quantified by sensitivity, specificity, negative and positive predictive value, and the C-statistic. A calibration plot was made to illustrate the agreement between expected risks and observed outcomes. Perfect calibration is characterized by a line with an intercept of 0 and a slope of 1.[17]

Results

Study population

The derivation cohort consisted of 255 patients, 10,638 observation days, compromising 1,319 hospital admissions. The median length of admission was one day (interquartile range (IQR) 1-23), reflecting the large number of day admissions. Thirty-eight percent of admissions was longer than one day, median 27 days (IQR 16-35). The median age of the patients was 56.9 (IQR 44.3-65.4), most were men (60.4%) and the majority was diagnosed with acute myeloid leukemia (74.1%) (table 1).

Table 1. Patient characteristics

	Complete derivation cohort	**Sample derivation cohort**
Patients	255	149
Male gender (%)	154 (60.4)	87 (58.4)
Age in years, median (IQR)	56.9 (44.3-65.4)	58.4 (44.9-67.2)
Diagnosis		
AML (%)	189 (74.1)	113 (75.8)
RAEB (%)	20 (7.8)	11 (7.4)
ALL (%)	46 (18.0)	25 (16.8)
Hospital admissions (n)	1319	265
Length of hospital stay, median (IQR)	1 (1-23)	25 (2-35)
Observation days	10,638	353
CT-scan (%)	75 (0.7)	75 (21.3)
Hemoglobin drop		
>0.8 to 1.6g/dl (%)	572 (5.4)	42 (11.9)
>1.6 to 1.9 g/dl (%)	29 (0.3)	20 (5.7)
≥1.9 to 2.2 g/dl (%)	49 (0.5)	22 (6.2)
≥2.2 to 2.8 g/dl (%)	18 (0.2)	18 (5.1)
≥2.8 g/dl (%)	13 (0.1)	13 (3.7)
Transfusion need		
2 products (%)	1,270 (11.9)	50 (14.2)
3 products (%)	1,126 (10.6)	43 (12.2)
4 products (%)	418 (3.9)	40 (11.3)
5 products (%)	156 (1.5)	31 (8.8)
≥ 6 products (%)	136 (1.3)	31 (8.8)
Control (%)	7216 (67.8)	90 (25.5)

Chart review was performed for a random sample of 353 hospitalization days (149 patients). The final sample contained more days with certain characteristics than would be expected solely based on the sampling scheme, since transfusion need and drop in hemoglobin are correlated (table 1).

Within the sample, 19 cases of major hemorrhage were found, corresponding to 16 unique patients. Of these, ten hemorrhages were intracranial, four gastro-intestinal, three following an invasive procedure, one pulmonary and one vaginal. None of the hemorrhages occurred during a day admission. Extrapolated to the complete cohort of 255 patients, 6.3% of patients experienced major hemorrhage, corresponding to an incidence of .22 per 100 hospitalization days. Including all grades of severity, 43 patients suffered from a bleeding event on 59 different days. Extrapolated to the complete cohort, the incidence of any hemorrhage was 8.4 per 100 hospitalization days.

Table 2. Univariable predictive capacity for major hemorrhage for CT-scan of the brain and several cut-off values of hemoglobin drop and transfusion need.

Variables	Sensitivity in % (CI)	Specificity in % (CI)	Positive predictive value in % (CI)	Negative predictive value in % (CI)	C-statistic (CI)
CT-scan brain	43.5 (23.2; 65.5)	99.4 (99.2; 99.5)	13.3 (6.6; 23.2)	99.9 (99.8; 99.9)	0.714 (0.61; 0.82)
Hemoglobin drop					
>0.8 g/dl	73.9 (51.6; 89.8)	94.5 (94.0; 94.9)	2.9 (1.7; 4.5)	99.9 (99.9; 100)	0.842 (0.75; 0.93)
≥1.6 g/dl	47.8 (26.8; 69.4)	99.2 (99.0; 99.4)	11.8 (6.1; 20.2)	99.9 (99.8; 99.9)	0.735 (0.63; 0.84)
≥2.0 g/dl	34.8 (16.4; 57.3)	99.4 (99.2; 99.5)	11.1 (4.9; 20.7)	99.9 (99.8; 99.9)	0.671 (0.57; 0.77)
≥2.4 g/dl	26.1 (10.2; 48.4)	99.8 (99.6; 99.8)	19.4 (7.5; 37.5)	99.8 (99.7; 99.9)	0.629 (0.54; 0.72)
≥2.8 g/dl	21.7 (7.5; 43.7)	99.9 (99.8; 100)	38.5 (13.9; 68.4)	99.8 (99.7; 99.9)	0.608 (0.52;0.69)
Transfusion need					
2 products	13.0 (2.8; 33.6)	88.0 (87.4; 88.7)	0.2 (0.05; 0.7)	99.8 (99.7; 99.9)	0.505 (0.44; 0.58)
3 products	4.4 (0.1; 21.9)	89.3 (88.7; 89.9)	0.1 (0.0; 0.5)	99.8 (99.6; 99.9)	0.468 (0.43; 0.51)
4 products	26.1 (10.2; 48.4)	96.6 (96.2; 96.9)	1.7 (0.6; 3.6)	99.8 (99.7; 99.9)	0.613 (0.52; 0.71)
5 products	4.4 (0.1; 21.9)	98.6 (98.4; 98.8)	0.7 (0.0; 3.7)	99.8 (99.7; 99.9)	0.515 (0.47; 0.56)
≥ 6 products	43.5 (23.2; 65.5)	98.9 (98.7; 99.1)	8.1 (4.0; 14.4)	99.9 (99.8; 99.9)	0.712 (0.61; 0.82)

Derivation cohort

Univariable analysis revealed that a hemoglobin drop of at least 0.8 g/dl and the need of six or more transfusions had the best discriminative capacity for major hemorrhage and for bleedings of all grades (table 2 and online supplements table

S2). Combined with the CT- brain (yes/no), the complete model had a C-statistic of 0.988 (confidence interval (CI) 0.981 to 0.995) for major hemorrhage and of 0.545 (CI 0.533 to 0.557) for all bleedings (figure 1). The coefficients of the model are depicted in the online supplements table S3. CT- brain or a combination of any of two indicators corresponded to a predicted risk of ≥0.02, with a sensitivity of 78.3% (CI 56.3 to 92.5) and a specificity of 99.2% (CI 99.1 to 99.4) (table 3). When at least one indicator is present (predicted risk ≥0.006), the sensitivity was 100% (CI 85.2 to 100) with a specificity of 93.1% (CI 92.6 to 93.5) (table 3). With an
incidence of 0.22 per 100 hospitalization days, 454.5 days have to be screened to detect one case. This is reduced to 5.5 days when a predicted risk of ≥0.02 is taken as cut off (table 3).

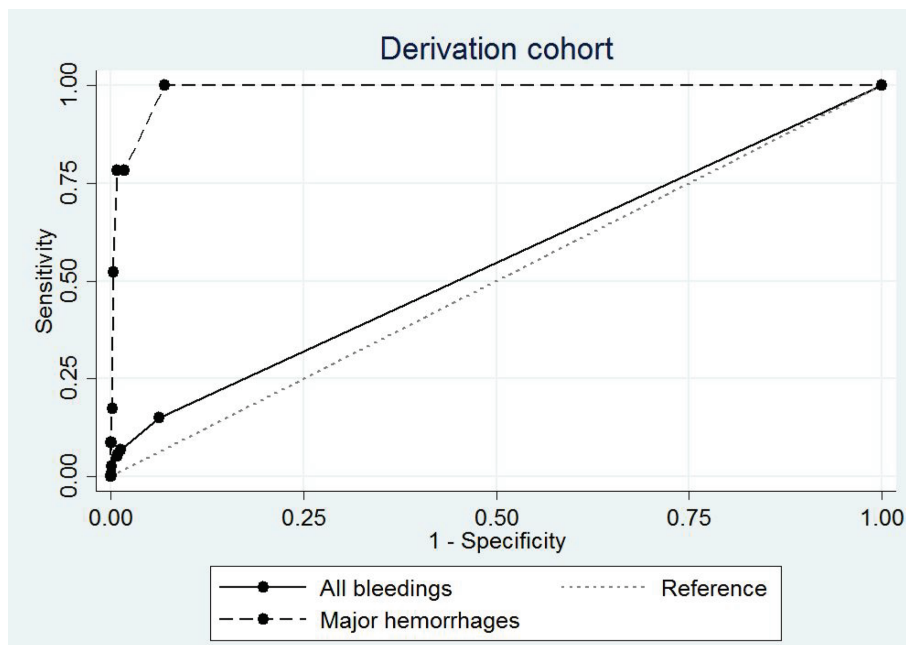
Table 3. Characteristics and performance of the model in the derivation cohort

Predicted risk*	CT†	Hb†	Tx†	Sensitivity in % (CI)	Specificity in % (CI)
All	0	0	0	100 (85.2; 100)	0 (0; 0.04)
≥0.006	0	+	0	100 (85.2; 100)	93.1 (92.6; 93.5)
≥0.013	0	0	+	78.3 (56.3; 92.5)	98.3 (98.1; 98.6)
≥0.022	+	0	0	78.3 (56.3; 92.5)	99.2 (99.1; 99.4)
≥0.250	0	+	+	52.2 (30.6; 73.2)	99.7 (99.6; 99.8)
≥0.362	+	+	0	17.5 (5.0; 38.8)	99.9 (99.8; 99.9)
≥0.538	+	0	+	8.7 (1.1; 28..0)	100 (99.9; 100)
≥0.967	+	+	+	8.7 (1.1; 28.0)	100 (100; 100)

Predicted risk*	Positive predictive value in % (CI)	Negative predictive value in % (CI)	Days needed to screen ‡	False negatives
All	0.2 (0.1; 0.3)	N/A§	454.5	0
≥0.006	3.1 (2.0; 4.6)	100 (100; 100)	34.7	0
≥0.013	9.3 (5.6; 14.3)	99.9 (99.9; 100)	11.0	5
≥0.022	18.4 (11.3; 27.5)	99.9 (99.9; 100)	5.5	5
≥0.250	27.9 (15.3; 43.7)	99.9 (99.8; 99.9)	3.6	11
≥0.362	20.0 (5.7; 43.7)	99.8 (99.7; 99.9)	5.1	19
≥0.538	50.0 (6.8; 93.2)	99.8 (99.7; 99.9)	2.0	21
≥0.967	100 (15.8; 100)	99.8 (99.7; 99.9)	1.0	21

*The sample was reweighted according to the distribution of the indicators in the complete cohort. The total number of events in reweighted dataset was 23. * The predicted risks include the risk for a given risk factor or larger risks (the lines below). †CT: CT scan brain, Hb: hemoglobin, Tx: transfusion.*
+ indicates presence and 0 indicates absence of the indicator. ‡ Calculated with an incidence of 0.22 per 100 days, which was the incidence in the extrapolated cohort. §N/A not applicable, negative predicted value can't be calculated when all days are screened.

Figure 1. ROC curve of the model in the derivation cohort



AUC for major hemorrhages was 0.988 (0.981: 0.995), for bleedings of all severity 0.545 (0.533: 0.557). The depicted results are derived from the sample and extrapolated to the entire cohort.

Validation cohort

The external validation total cohort consisted of 436 patients, 19,188 hospitalization days, compromising 1,276 hospital admissions. The median length of admission was 17 days (IQR 2-32.5). In contrast to the hospital of the derivation cohort, day admissions were differently coded and therefore not included in the database. The median age of the patients was 57.7 year (IQR 46.0- 65.5), 58.7% were men and 74.5% were diagnosed with acute myeloid leukemia (table 4). The patient characteristics stratified by hospital are depicted in the online supplements table S4.

Chart review was performed for 599 hospitalization days (294 patients). For 17 days (9 patients) no information about bleeding could be retrieved from the patient files. These days were excluded from all analyses. Within the remaining 582 days (291 patients), 42 patients experienced major hemorrhage on 52 different days. Extrapolated to the complete cohort, this corresponded to an incidence of 0.46 per 100 hospitalization days. Assuming that all major hemorrhages were detected by

using this model, 9.6% of the patients experienced major hemorrhage in the complete cohort. Seventeen were intracranial, seventeen gastro-intestinal, six urogenital, four followed an invasive procedure, three hemorrhages derived from the spleen, three patients had an epistaxis requiring a red blood cell transfusion, one patient had a pleural hemorrhage and one had a retina bleeding event with visual impairment.

Table 4. Baseline characteristics validation cohort

	Validation cohort	**Sample validation cohort**
Patients	436	294
Male gender (%)	256 (58.7)	174 (59.2)
Age in years, median (IQR)	57.7 (46.0-65.5)	56.7 (40.5-65.4)
Diagnosis		
AML (%)	325 (74.5)	216 (73.5)
RAEB (%)	28 (6.4)	21 (7.1)
ALL (%)	83 (19.0)	55 (18.7)
Hospital admissions (n)	1,276	458
Length of hospital stay, median (IQR)	17 (2-32.5)	27 (10-37)
Observation days	19,188	599
CT-scan (%)	110 (0.57)	110 (18.4)
Hemoglobin drop		
>0.8 to 1.6g/dl (%)	1,293 (6.7)	203 (33.9)
>1.6 to 1.9 g/dl (%)	103 (0.5)	14 (2.3)
≥1.9 to 2.2 g/dl (%)	145 (0.8)	25 (4.2)
≥2.2 to 2.8 g/dl (%)	89 (0.5)	11 (1.8)
≥2.8 g/dl (%)	45 (0.2)	11 (1.8)
Transfusion need		
2 products (%)	1,159 (60)	81 (13.5)
3 products (%)	1,040 (5.4)	50 (8.4)
4 products (%)	656 (3.4)	14 (2.3)
5 products (%)	147 (0.8)	7 (1.2)
≥ 6 products (%)	51 (0.3)	92 (15.4)
Control (%)	56 (0.3)	400 (66.8)

Table 5. Performance of the model in the external validation cohort

Predicted risk	Sensitivity in % (CI)	Specificity in % (CI)
All	100 (95.8; 100)	0 (0; 0.02)
≥0.006	100 (95.8; 100)	90.7 (90.2; 91.1)
≥0.013	54.0 (43.0; 64.8)	99.2 (99.1; 99.3)
≥0.022	41.4 (30.9; 52.4)	99.4 (99.3;99.5)
≥0.250	29.9 (20.5; 40.6)	99.8 (99.7; 99.9)
≥0.362	8.1 (3.3; 15.9)	99.9 (99.9; 99.9)
≥0.538	3.5 (0.7; 9.8)	100 (100; 100)
≥0.967	2.3 (0.3; 8.1)	100 (100; 100)

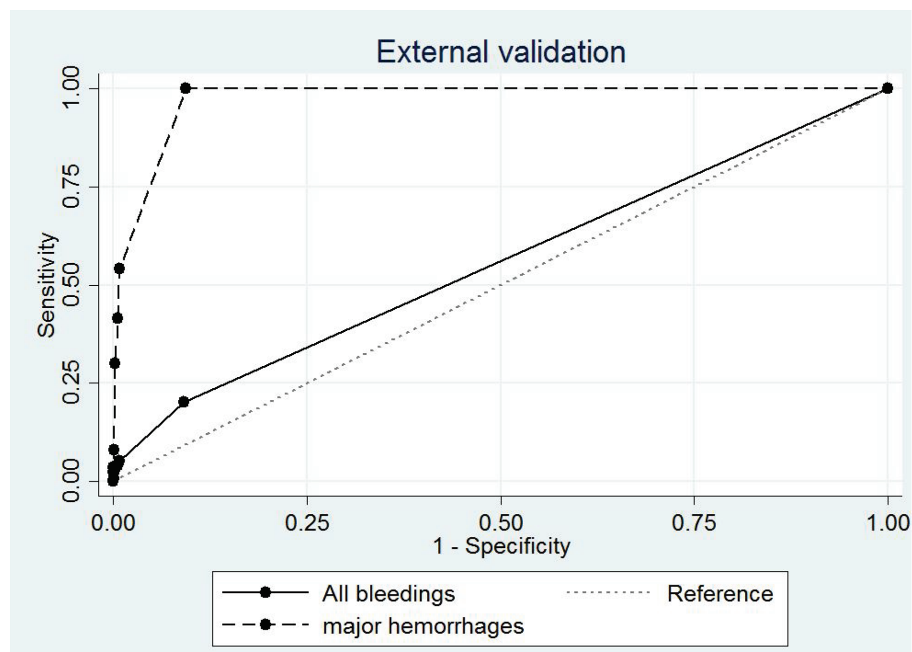
Predicted risk	Positive predictive value in % (CI)	Negative predictive value in % (CI)	Days needed to screen*	False negatives
All	0.5 (0.4; 0.6)	N/A†	217.4	0
≥0.006	4.7 (3.8; 5.7)	100 (100; 100)	23.6	0
≥0.013	24.4 (18.5; 31.0)	99.8 (99.7; 99.8)	4.2	40
≥0.022	24.3 (17.7; 32.1)	99.7 (99.6; 99.8)	4.2	51
≥0.250	41.9 (29.5; 55.2)	99.7 (99.6; 99.8)	2.4	61
≥0.362	29.2 (12.6; 51.1)	99.6 (99.5; 99.7)	3.5	80
≥0.538	100 (29.2; 100)	99.6 (99.5; 99.6)	1	84
≥0.967	100 (15.8; 100)	99.6 (99.4; 99.6)	1	85

*The sample was reweighted according to the distribution of the indicators in the complete cohort. The total number of events in the reweighted dataset was 87. * Calculated with an incidence of 0.46 per 100 days, which was the incidence in the extrapolated cohort. † N/A not applicable, negative predicted value can't be calculated when all days are screened.*

For a predicted risk of ≥0.02, the sensitivity of the model was 41.4% (CI 30.9 to 52.4), the specificity 99.4% (CI 99.3 to 99.5), and the days needed to screen 4.2. When at least one indicator was present (predicted risk ≥0.006) the sensitivity was 100% (CI 95.8 to 100), the specificity 90.7% (CI 90.2 to 91.1) and 23.6 days had to be screened to detect one case of major hemorrhage (table 5 and online supplements table S5). The C-statistic of the model was 0.975 (CI 0.970;980) (figure 2). Calibration of the model is shown in the online supplements, figure S1.

Including all grades of severity, 65 patients suffered from a bleeding event on 83 different days. This corresponded to an incidence of 5.5 bleedings per 100 hospitalization days, or 2.4 bleedings per patient in the complete cohort. The C-statistic of the model for all bleedings was 0.557 (CI 0.544; 0.569) (figure 2).

Figure 2. ROC curve for major hemorrhages and all bleedings in the external validation cohort



AUC for major hemorrhages was 0.975 (0.970: 0.980), for bleedings of all severity 0.557 (0.544: 0.569). The depicted results are derived from the sample and extrapolated to the entire cohort.

Discussion

Routinely recorded data can be used to accurately identify cases of major hemorrhages, WHO grade 3 and 4, among patients with acute leukemia. A model based on drop in hemoglobin ≥0.8 g/dL, the need of ≥6 transfusions and CT-brain allows the capture of cases with major hemorrhages in large datasets over a long follow-up period while minimizing costs and effort. The model has poor discriminative capacity for bleedings of all grades of severity.

Cases identified with this model can be used as an outcome regarding studies investigating risk factors for bleeding in large populations or to identify cases for a case control study. The average incidence in all cohorts combined was 0.37 per 100 hospitalization days. This implies that 270 days have to be screened to find one case of major hemorrhage. When at least one of the indicators is present, the days to

screen is limited to 34.7 to 23.1 days, without missing a single case. This could even be reduced to only 11 to 4.2 days by choosing a higher cut off risk, although with this strategy 40 of 87 (45.9%) cases will be missed. These are predominantly renal, gastrointestinal, and splenic hemorrhages, whereas all cases with intracranial bleeding will still be detected.

An advantage of routinely collected data is that it offers the opportunity to include larger populations which maximizes the generalizability. Additionally, patients in trials are mostly selected using rigorous in- and exclusion criteria which cannot be extrapolated to general practice [3]. A drawback of routinely collected data is that these are not collected for research purposes and therefore potentially more at risk for errors and missing data [18,19]. The accuracy and completeness of these data has been demonstrated by linking 99% of fatal events of the West of Scotland Coronary Prevention Study (WOSCOPS) trial to routinely collected ICD codes [20,21]. In addition, the incidence in our sample is comparable with the incidences reported in literature [11,22,23]. In the external validation cohort, we detected major hemorrhage among 9.6% of the patients, corresponding to an incidence of 0.46 per 100 days. A trial of 600 leukemia patients reported an incidence of 0.05 per 100 observation days.[23] In an observational study, the incidence was 5 out of 68 patients (7.8%) and in another trial this was 28 out of 255 patients (11%) [11,22].

In the current study, major hemorrhage was not reported in a standardized way and patients were not stringently observed. Instead, we used proxies for major blood loss and intracranial bleed. Limitation of this approach is that cases with retinal bleed with visual impairment (WHO grade 4) will be missed. In addition, patients have to survive long enough after start of hemorrhage to reach the threshold of hemoglobin drop or transfusion need, or a CT-scan. Therefore the model could underestimate the true incidence of major hemorrhage. However, we assume this does not outweigh the benefits of including all patients leading to a considerable increase in sample size.

Algorithms are often based on coding sets used in specific datasets, like the ICD codes. These are prone to changes in coding or medical practice and regional and temporal variation exists.[24] In contrast to these algorithms, we included variables that are easily accessible and less prone to variation. Calibration of the model in the external validation was imperfect. However, this model is not aimed to predict risks, but primarily to discriminate. Discriminative capacity of the model was very good in the derivation cohort as well as in the external validation cohort, which confirms the overall generalizability of this model.

In conclusion, we developed and validated a model based on routinely collected clinical data to reliably identify patients with major hemorrhage. This model will have particular significance for researchers and blood services who aim to investigate major hemorrhage among hematological patients with sufficient sample size, by limiting the number of days to screen.

Supplementary material

Supplementary material is available from the author upon request.

Table S1. WHO bleeding score, with specifications as used in the PlaDo trial
Table S2. Predictive capacity for CT-scan of the brain and several cut-off values of hemoglobin drop and transfusion need for bleeding of all severity.
Table S3. Beta's of the model
Table S4. Patients characteristics of the external validation cohort, stratified by hospital.
Table S5. Performance of the model in the external validation cohort stratified by hospital.
Figure S1. Calibration plot external validation

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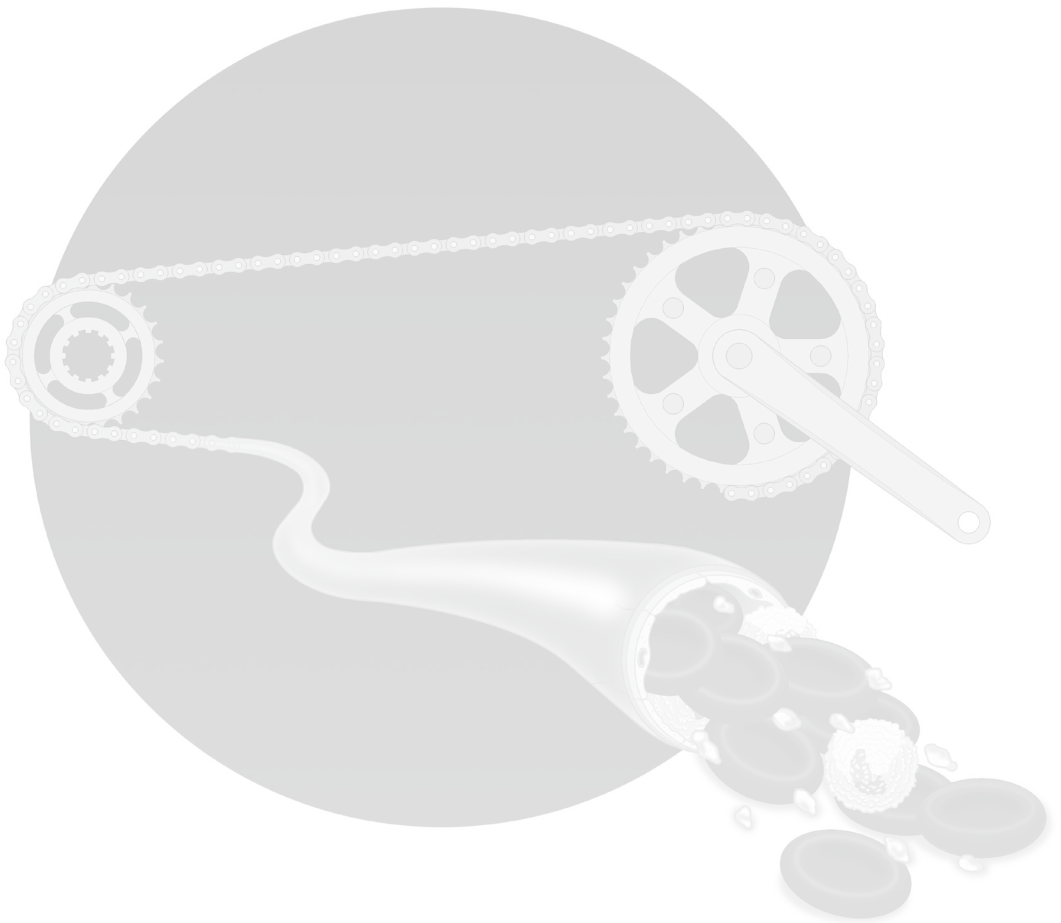

Chapter 5

Effect of platelet storage time on platelet measurements: a systematic review and meta-analyses

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Abstract

Background

The storage time of platelet products negatively affects bacterial safety and platelet function. However, low maximum storage time increases outdating of valuable products. Thus, to quantify the effect of platelet storage time on platelets measurements after platelet transfusion a systematic review and meta-analyses were performed.

Methods

Reports and meeting abstracts of randomized trials and observational studies, performed in humans, reporting platelets measurements after transfusion of platelet products of different storage times were selected until February 2016. Meta-analyses were performed for four different storage time contrasts, each answering a different question. Random effects models were used to account for substantial heterogeneity and the weighted mean differences were calculated.

Results

Our search strategy yielded 4,234 studies of which 46 papers satisfied the inclusion criteria. As judged by the 1 hour corrected count increment, transfusion of fresher platelets compared to stored platelets showed better increment. The weighed mean difference varied from 2.11 (95%CI: 1.51 to 2.71) to 2.68 (95%CI: 1.92 to 3.45). For the 24 hour corrected count increment the weighted mean difference varied from 1.36 (95%CI: 0.12 to 2.60) to 1.68 (95%CI: 1.07 to 2.28) depending on the contrast. Recovery and survival of old platelets as percentage of fresh platelets were 81% and 73% for the original definition contrast. For the extended storage contrast recovery and survival were 75% and 68%.

Conclusions

Fresh platelets were superior to old platelets for all platelets measurements and for all storage time contrasts meta-analyzed.

Introduction

Many papers have been published relating storage time of blood products to clinical outcomes and measurements. However, most of these focus on red blood cells.[1-9] Platelets are essential for hemostasis. Patients with thrombocytopenia or thrombocytopathy, due to hematologic malignancies, other blood disorders, bleeding, or medication, require platelet transfusions to prevent or treat bleeding.[6,7] The storage time of platelet products negatively affects bacterial safety and platelet function.[8,9] However, low maximum storage time increases outdating of valuable products. The balance between avoiding wastage and maintaining product safety and quality determines optimal storage time.[10] Maximum storage of platelets can be three to seven days, depending on the local or national guidelines and the type of product. For example, maximum storage time is three days in Japan[11], four days in Germany[12], and five days in the United States[13] and Brazil.[14] In The Netherlands, platelet products can be stored for a maximum of seven days.[15] As blood banks world-wide seek to increase maximum storage times, seven days storage will become more common. The effect that seven days storage has on product quality and safety will therefore become ever more important. In 2014 the Food and Drug Administration issued a draft guidance on safety testing and, during their 2015 annual meeting, the American Association of Blood Banks hosted a dedicated session “Paving the Way Towards Implementation of 7 Day Platelets”. Several studies have investigated the effect of storage time of platelets on platelets measurements and other outcomes.[16,17] However, no comprehensive systematic summary and quantification (meta-analyses) of the available evidence has been made to date. The objective of this systematic review and meta-analyses was to quantify the effect of platelet storage time on platelets measurements after platelet transfusion.

Methods

Search strategy

As pre-specified in the study protocol (supplemental material, appendix 1), we performed a systematic review to identify all randomized clinical trials and observational studies reporting storage time of platelets products. Potentially relevant papers and meeting abstracts were identified using MEDLINE (PubMed), EMBASE, Cochrane, CINAHL, Academic Search Premier, ScienceDirect and Web of Science databases until February 2016. No restriction on study design, language or year of publication was used (supplemental material appendix 2). Non-English papers were translated by native (Chinese and German) or fluent (Russian) speakers.

Study selection

Two reviewers independently reviewed, titles and abstracts to select studies reporting platelets storage time and platelets measurements. Pre-specified inclusion criteria were: (i) human: papers reporting exclusively animal studies were excluded; (ii) platelet product transfusion: papers that were exclusively about other blood products or about endogenously produced platelets were excluded; (iii) clinical (performed in patients or volunteers): in vitro, ex vivo, laboratory experiments, and simulation studies were excluded; (iv) storage time: reported as a variable in the paper; (v) original: letters, comments, and reviews not containing any original data were excluded; (vi) platelets measurements: papers that reported at least one of the five platelets measurements (count increment [$\times 10^9$/L]: pre-transfusion platelet count subtracted from post-transfusion platelet count;[16] corrected count increment [/dm]: count increment corrected for body surface area and platelet product dose;[16] recovery: proportion of platelets recovered from the circulation;[17] survival: mean residual life span;[17] and half-life) and (vii) data necessary for meta-analyses reported: point estimate (i.e. mean or median) and measure of precision (i.e. standard deviation, standard error, interquartile range or range).

Disagreements between reviewers were discussed with a third reviewer. Papers were included for full text assessment if no decision was possible on title and abstract alone.

Full text papers were reviewed again for all inclusion criteria. Papers were excluded if the data presented were the same (totally or partially) as those presented in another selected paper. In this case papers were preferred over meeting abstracts and chronologically newer papers were preferred over older ones.

Risk of bias assessment

The risk of bias was evaluated using “The Cochrane Collaboration’s tool for assessing risk of bias” to evaluate randomized clinical trials, and the “Fowkes & Fulton tool” to evaluate both randomized clinical trials and observational studies.18-20 The items in the Fowkes & Fulton tool are appropriate study design, representative study sample, acceptable control group, quality of measurements and outcome, completeness, and confounding, which are similar to the ACROBAT NRSI Cochrane tool for assessing non-randomized studies.[21] For the randomized studies there was perfect agreement between the two tools. Papers with high risk of bias in any of the assessed domains of bias were excluded from the final selection.

Storage time definition

For simplicity only the terms 'fresh' and 'old' are used throughout this paper. The term 'fresh' is used to refer to the storage time group stored for a shorter time than its comparator group (in the same paper). Common synonyms for 'fresh' used in the literature include 'new' and 'young'. The term 'old' is used to refer to the storage time group with the longer storage time. Common synonyms for 'old' include 'stored' and 'aged'.

Storage time comparisons

To answer different questions regarding the effect of storage time of platelets results were meta-analyzed in four different ways.[22] If a paper did not report the results in a way compatible with dichotomizing the data according to one of these definitions, that paper was excluded from that particular analysis.

a) Original definition (as reported): Fresh and old were included in the meta-analysis as reported in the paper. If a paper's results were not presented in two groups the results were dichotomized into fresh if stored ≤3 days and old if stored ≥4 days.
b) Maximum storage 5 days (0-2 vs. 3-5): Papers were included that reported results for zero to two days (fresh) and three to five days (old). This analysis provides a clinically relevant answer to the question whether platelets on the 'fresh half' of the storage time spectrum are different from those on the 'old half', for the very common situation where the maximum storage time is five days
c) Extreme difference (0-2 vs. 5-7): To examine the effect of extreme differences in storage time only papers were included if they reported results for zero to two days (fresh) and five to seven days (old). This analysis provides the strongest contrast and therefore is the most sensitive indication whether any effect exists or not.
d) Extended storage (0-5 vs. 6-7): In this analysis papers were included that reported results for zero to five days (fresh) and for six or seven days (old). This analysis compares 'standard maximum storage' of five days directly to 'extended storage' till seven days. It is therefore most relevant to the situation where extended storage is either allowed, or under consideration for implementation.

Each one of these four meta-analyses was performed independently. For all analyses a minimum of five papers (per platelets measurement) was required to estimate the pooled effect. Clinical measurements reported in less than five papers

were reported in the selection flowchart (figure 1), but were not included in the meta-analyses. Moreover, for all analyses, results from storage time beyond normal blood banking practice (i.e. >7 days) were disregarded. Pooled effects are presented per platelets measurement.

Data extraction

As specified in the study protocol (online appendix 1), all relevant data reported in the papers were first recorded exactly as reported and subsequently organized and recalculated as described below. Products were grouped into four product groups: apheresis platelets stored in plasma (apheresis plasma), buffy-coat derived platelets stored in plasma (BC plasma), platelet rich plasma (PRP), and buffy-coat derived platelets stored in platelet additive solution (BC PAS). To allow pooling of the data, the original results sometimes needed to be recalculated or transformed:

a) If the standard error of the mean (SEM) was reported, the standard deviation (SD) was calculated: $SD = SEM * \sqrt{n}$;
b) Mean and standard deviation were calculated from medians, ranges and quartiles,[23] since a normal distribution could be expected to be the true underlying distribution from which sampling took place. Only six out of 46 studies did not report their results as normally distributed. We therefore assumed those six were not sufficiently confident of a normal distribution based on their own results alone. Based on the other 40 studies, all sampling from the same underlying distribution, and all reporting a normal distribution, we could be more confident than any individual study;
c) Similar products (i.e. differences in post-production processing) were merged using standard formulas for combining samples sizes (Σn_i), means ($\sum \overline{x_i} * n_i / (\sum n_i)$) and standard deviations ($SD = (\Sigma(n_i - 1)s_i^2 / \Sigma(n_i - 1))^{½}$) from multiple groups. Whereas really different products (i.e. different donation procedure or storage medium) presented in the same paper were not merged;
d) When necessary originally reported categories were merged into the four different definitions of fresh versus old using standard formulas, as described above (item c);
e) Results presented in hours were recalculated to days;
f) Platelets measurements reported between zero and four hours after transfusion were considered ‘1 hour’; platelets measurements reported between eight and 28 hours after transfusion were considered ‘24 hours’.

Analyses

Results were pooled across studies using random effects methods to account for substantial heterogeneity, as indicated by high I^2-values. Weighted mean differences, also known as non-standardized mean differences, were calculated for continuous outcomes. Heterogeneity between studies was assessed using the I^2 statistic. The I^2 value ranges from 0% to 100% and calculates the proportion of variation due to heterogeneity rather than due to chance. Reporting (or publication) bias was analyzed using a funnel plot and its asymmetry was assessed using Egger's test.[24] All outcomes (i.e. parameters) were transformed to the same scale to allow the construction of a single funnel plot for all platelets measurements combined. The standardized model was therefore used in this analysis (i.e. as opposed to the non-standardized model used to report the main effects) and all studies were centered around the null effect by subtracting the standardized mean differences per platelets measurement.

Recovery and survival were expressed as percentage recovery and survival achieved with old platelets, compared to fresh platelets. This provides some insight into the order of magnitude of difference to expect, since it allows comparison to the requirements of the Food and Drug Administration (FDA). The FDA requires a minimum of 67% for recovery and 58% for survival, compared to day zero platelets, for any type of platelet product or production process to be allowed into platelets use.[13]

Additional analyses

Additional analyses were performed to clarify whether observed heterogeneity could potentially be attributed to effect modification. Explored possible underlying differences included differences in outcomes, storage times contrasts (analyses a to d), product types, studies populations, and studies design: (i) funnel plot for each outcome separately; (ii) forest plots for each outcome separately and stratified by different product types and different populations; and (iii) summary mean difference according to whether the study was randomized or not.

Results

Selection

The search retrieved 4,234 records. 4,099 records were excluded because they were: an exclusively animal study (199); not about platelet transfusions (1521); not *in vivo* or did not report a platelets outcome (1077); not about storage time (234); did not present original data (196); or because the titles were irrelevant (872 from

the 886 records which abstracts were not available). Upon full text review of the remaining 135 papers a further 48 were excluded because of the above mentioned exclusion criteria (n=32), or because of high risk of bias (n=16, mostly because the fresh and old groups also differed in other respects like storage medium, type of storage bag, storage conditions, type of donation, or production process). Further nine papers were excluded because their data were presented in another selected paper, 19 because they did not report any platelets measurement and 13 because they did not report the data necessary for the meta-analyses. The final selection included 46 papers, 13 randomized trials and 33 observational studies (figure 1). The complete list of selected papers and their qualitative overview can be found in the supplemental material (appendix 3). Only six papers failed to report normally distributed results. To allow pooling the data their results were recalculated (see methods section for details).

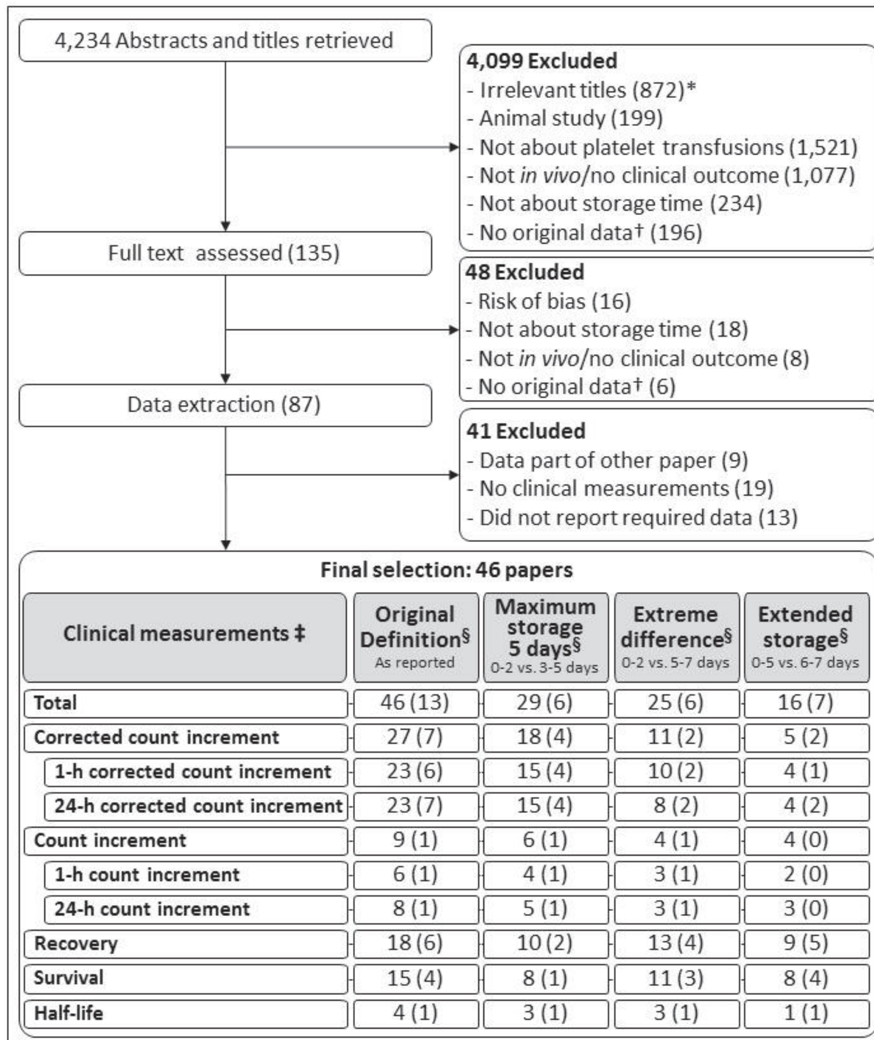
Reported outcomes

Of the 46 selected papers, 27 papers reported corrected count increments (23 reported the 1 hour and 23 reported the 24 hour corrected count increment). Nine papers reported count increment (six papers reported 1 hour and eight reported 24 hour count increment). Eighteen papers reported platelet recovery. Survival was reported in 15 papers and half-life was reported in four (figure 1).

Meta-analyses

Figure 2 shows the funnel plot for all outcomes combined. There is a relative lack of smaller studies (i.e. larger standard error) favoring older platelets, compared to either smaller studies favoring fresh platelets or larger studies. This indicates a bias towards withholding publication of small and therefore statistically unreliable studies showing a benefit of older platelets. Publication bias was present as indicated by Egger's bias coefficient 2.14 (95% confidence interval (CI): 1.59 to 2.70). Half-life did not reach the cut-off of a minimum of five papers and was therefore not included in any of the meta-analyses.

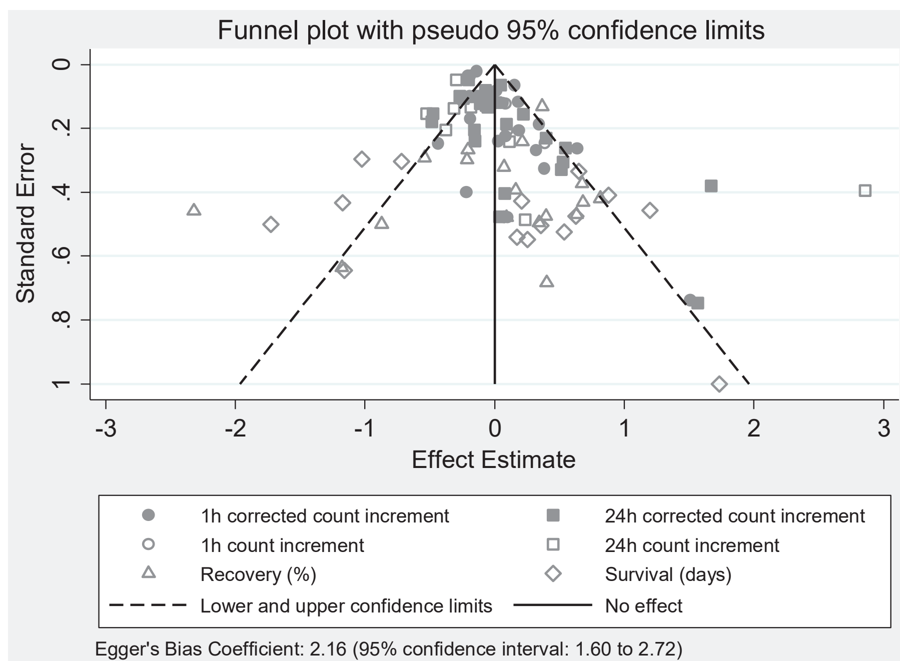
Figure 1. Flowchart study selection



Clinical measurements ‡	Original Definition§ As reported	Maximum storage 5 days§ 0-2 vs. 3-5 days	Extreme difference§ 0-2 vs. 5-7 days	Extended storage§ 0-5 vs. 6-7 days
Total	46 (13)	29 (6)	25 (6)	16 (7)
Corrected count increment	27 (7)	18 (4)	11 (2)	5 (2)
1-h corrected count increment	23 (6)	15 (4)	10 (2)	4 (1)
24-h corrected count increment	23 (7)	15 (4)	8 (2)	4 (2)
Count increment	9 (1)	6 (1)	4 (1)	4 (0)
1-h count increment	6 (1)	4 (1)	3 (1)	2 (0)
24-h count increment	8 (1)	5 (1)	3 (1)	3 (0)
Recovery	18 (6)	10 (2)	13 (4)	9 (5)
Survival	15 (4)	8 (1)	11 (3)	8 (4)
Half-life	4 (1)	3 (1)	3 (1)	1 (1)

** 886 titles screened (abstracts not available);*
† letters/comments/reviews/reports;
‡ more than one possible outcome per paper
§ between brackets the number of 'randomized trials'

Figure 2. Funnel plot



a) Meta-analysis: original definition (as reported)

Figure 3a shows the pooled weighted mean differences of fresh platelets minus old platelets. Pooled effect estimates were: 1 hour corrected count increment 2.30 (CI: 1.72 to 2.88); 24 hour corrected count increment 1.68 (CI: 1.07 to 2.28); 1 hour count increment 4.47 (CI: 2.13 to 6.82); 24 hour count increment 4.60 (CI: 0.73 to 8.47); recovery 11.12% (CI: 7.80% to 14.43%); survival 2.08 days (CI: 1.63 to 2.52). The I^2 ranged from 53% to 92% (table 1 and figure 3a). Based on the pooled means and standard deviation, recovery of old platelets was 81% of fresh platelets and survival of old platelets was 73% of fresh platelets (table 1).

b) Meta-analysis: maximum storage 5 days (0-2 vs. 3-5 days)

Twenty-nine papers were included in this analysis, 18 papers reported corrected count increment (15 the 1 hour corrected count increment, and 15 the 24 hour corrected count increment) and six reported count increment (four the 1 hour count increment, and five the 24 hour count increment). Recovery and survival were reported in ten and eight papers.
The pooled weighted mean differences estimated for fresh minus old were: 1 hour

corrected count increment 2.11 (CI: 1.51 to 2.71); 24 hour corrected count increment 1.36 (CI: 0.12 to 2.60); 24 hour count increment 4.69 (CI: 0.41 to 8.96); recovery 7.41% (CI: 1.53% to 13.28%) and survival 1.59 days (CI: 1.01 to 2.17). I^2 ranged from 45% to 90% (table 1 and figure 3b). Recovery and survival of old platelets were 88% and 80% of fresh platelets (table 1).

c) Meta-analysis: extreme difference (0-2 vs. 5-7 days)

Twenty-five papers were included in the extreme difference (0-2 vs. 5-7 days) meta-analyses. Ten papers reported corrected count increment as an outcome (11 the 1 hour corrected count increment and eight the 24 hour corrected count increment). Four papers reported count increment (three the 1 hour count increment and three the 24 hour count increment). Recovery, and survival were reported in 13 and 11 papers (figure 1).

Figure 3c shows the pooled weighted mean differences for fresh minus old for corrected count increment, recovery and survival. Count increment did not reach the cut-off of a minimum of five papers. Pooled effect estimates were: 1 hour corrected count increment 2.68 (CI: 1.92 to 3.45); 24 hour corrected count increment 1.36 (CI: 0.08 to 2.63); recovery 12.71% (CI: 7.63% to 17.80%); and survival 2.30 days (CI: 1.76 to 2.84). The I^2 ranged from 46% to 81% (table 1 and figure 3c). Recovery of old platelets was 80% of fresh and survival was 71% (table 1).

d) Meta-analysis: extended storage (0-5 vs. 6-7 days)

Sixteen papers compared standard storage (0-5 days) to extended storage (6-7 days). Nine papers reported recovery and eight papers reported survival as an outcome. Corrected count increment and count increment did not reach the cut-off of a minimum of five papers. The pooled weighted mean differences for fresh minus old were: recovery 15.44% (CI: 10.22% to 20.66%) and survival 2.48 days (CI: 1.86 to 3.09). The I^2 were 70% and 72% (table 1 and figure 3d). Recovery and survival of old platelets were 75% and 68% of fresh platelets (table 1).

Table 1. Mean differences in platelets measurements after transfusion of fresh and old platelets products according to four different definitions of fresh and old.

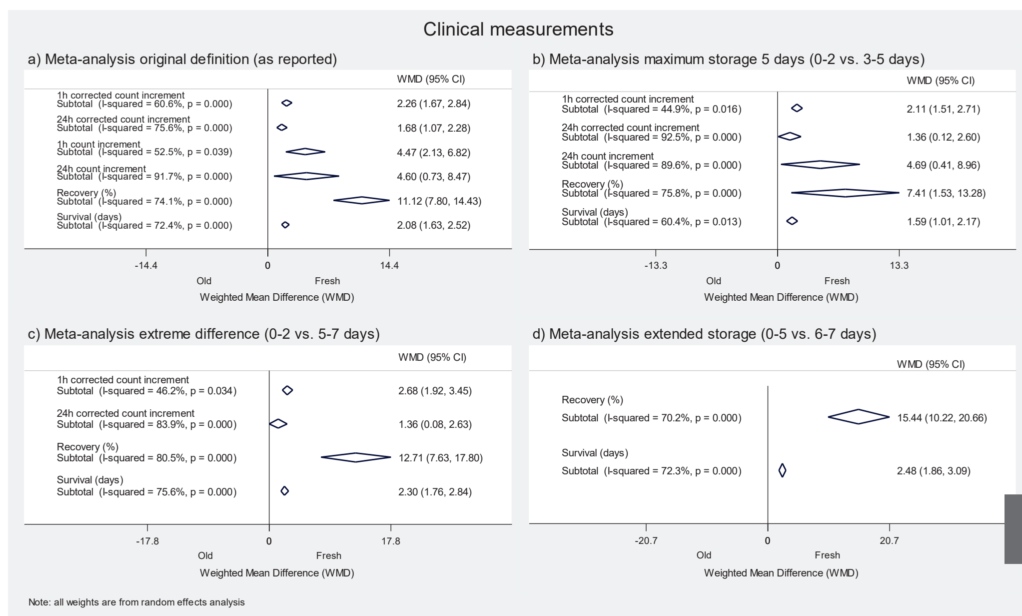
	Original definition as reported	Maximum storage 5 days 0-2 vs. 3-5 days	Extreme difference 0-2 vs. 5-7 days	Extended storage 0-5 vs. 6-7 days
1h corrected count increment	2.30 (1.72 to 2.88)	2.11 (1.51 to 2.71)	2.68 (1.92 to 3.45)	-
24h corrected count increment	1.68 (1.07 to 2.28)	1.36 (0.12 to 2.60)	1.36 (0.08 to 2.63)	-
1h count increment	4.47 (2.13 to 6.82)	-	-	-
24h count increment	4.60 (0.73 to 8.47)	4.69 (0.41 to 8.96)	-	-
Recovery (%) old as % of fresh*	11.12 (7.80 to 14.43) 81%	7.41 (1.53 to 13.28) 88%	12.71 (7.63 to 17.80) 80%	15.44 (10.22 to 20.66) 75%
Survival (days) old as % of fresh*	2.08 (1.63 to 2.52) 73%	1·59 (1.01 to 2.17) 80%	2.30 (1.76 to 2.84) 71%	2.48 (1.86 to 3.09) 68%

Values are weighted mean differences fresh minus old (95% confidence interval) or percentages (%)
**old as percentage of fresh*

<u>Additional analyses</u>

The supplemental material shows funnel plot for each outcome separately and complete forest plots for each outcome separately, stratified by different product types and different populations. It also presents summary mean difference according to whether the study was randomized or not and the underlying distribution (absolute numbers) of the weighted mean differences (appendix 4 and 5). All results were similar to the overall pooled results as presented in the main text, table, and figures.

Figure 3. Summary mean differences between fresh and old platelet products in platelet measurements according to four different definitions of old and fresh.



Heterogeneity, as indicated by I^2 values, was typically much lower in analyzed subgroups, especially upon stratification by product type. This indicates product type to be a source of heterogeneity. However, since overall pooled results were very similar to pooled subgroup results, overall results can be used as summary measures. Subgroup results are therefore only reported in the supplemental material, appendix 4.

Discussion

Fresher platelets were superior to older platelets for all platelets measurements and all different storage time contrasts investigated. Strengths of this study include the comprehensiveness. There were no limitations on the type of outcome, publication date, study design, population, and language. Also, search keywords were defined very broadly, including as many papers as possible. The search strategy was applied to many different literature databases and queries for all databases were built by a senior librarian, specialized in performing systematic literature searches. This approach likely ensured that all potentially relevant papers were retrieved.

From all selected papers the maximum possible amount of available data were retrieved. Data reported in ways that did not allow pooling (e.g. medians and ranges or interquartile ranges), were recalculated into means and standard deviations, which do allow pooling. Data were extracted from graphs when necessary. Therefore, we were able to pool the results and perform the meta-analyses on data from as many papers as possible.

Another important strength of this study is the quality of included data. Risk of bias was assessed in two different ways and we found perfect agreement between the two assessment tools. Out of 135 studies reporting at least one platelets measurement 16 were excluded based on the risk of bias assessment. Of the remaining studies data that allowed for pooling of results in the meta-analyses could be extracted from 46.

A possible limitation is that not enough randomized trials were included to perform a meta-analysis restricted to randomized trials. However, to have full transparency of our reporting, we showed results stratified between randomized trials and non-randomized trials in the supplemental material. All results in these analyses were in the same direction and in the same magnitude as those presented in the main text.

Another remark to be made is about the high heterogeneity between the studies measured as I^2. As recommended by The Cochrane, besides verifying the data and exploring the heterogeneity, a random-effects meta-analysis was performed.[25]

We found indications of the presence of publication bias. The funnel plot shows a slight preference for smaller studies favoring fresher platelets and Egger's bias coefficient also indicates the presence of publication bias. However, the funnel plot is centered around zero by subtracting the standardized mean effect. Therefore, the largest observed ‘negative effect’, is in reality still an effect in favor of fresher platelets. Thus, although publication bias may have had a minor effect on the size of our effect estimates, it seems unlikely that this could have materially influenced our conclusions.

These potential consequences of transfusing older platelets, however, have to be put in perspective relative to the consequences of supplying exclusively fresher platelets. The Dutch blood supply organization (Sanquin) switched to extended storage of platelets (i.e. maximum storage of seven days instead of five) in 2002. This prolongation of storage time reduced outdating from 20% to about 10%, reducing cost and increasing platelet availability.[26]

In conclusion, our results indicate that fresh platelets are more likely to result in a successful transfusion than old platelets. With successful transfusion defined as a count increment based measurement being above a specific threshold. However, as currently judged by means of a corrected count increment, the success of a transfusion results from a mixing of effects of patient and product related factors. To be clinically relevant the judgment of success of a transfusion should depend on patient related factors only and be separated from product related factors as much as possible. So besides body surface area and platelet dose of the product, storage time should also be taken into account, to arrive at an even better corrected count increment to judge the success of transfusions. We therefore recommend more research into a storage time independent measure for the success of a platelet transfusion.

Supplemental material

Available at http://onlinelibrary.wiley.com/doi/10.1111/vox.12443/full#footer-support-info

Appendix S1. Protocol
Appendix S2. Search strategy: queries
Appendix S3. Reference list and qualitative overview – Included papers
Appendix S4. Funnel plot per outcome; forest plots of weighted mean differences per outcome, product group and population (patients/volunteers); and Summary mean differences according to study design (RCT/Non-RCT)
Appendix S5. Underlying distributions (absolute numbers) of the weighted mean differences

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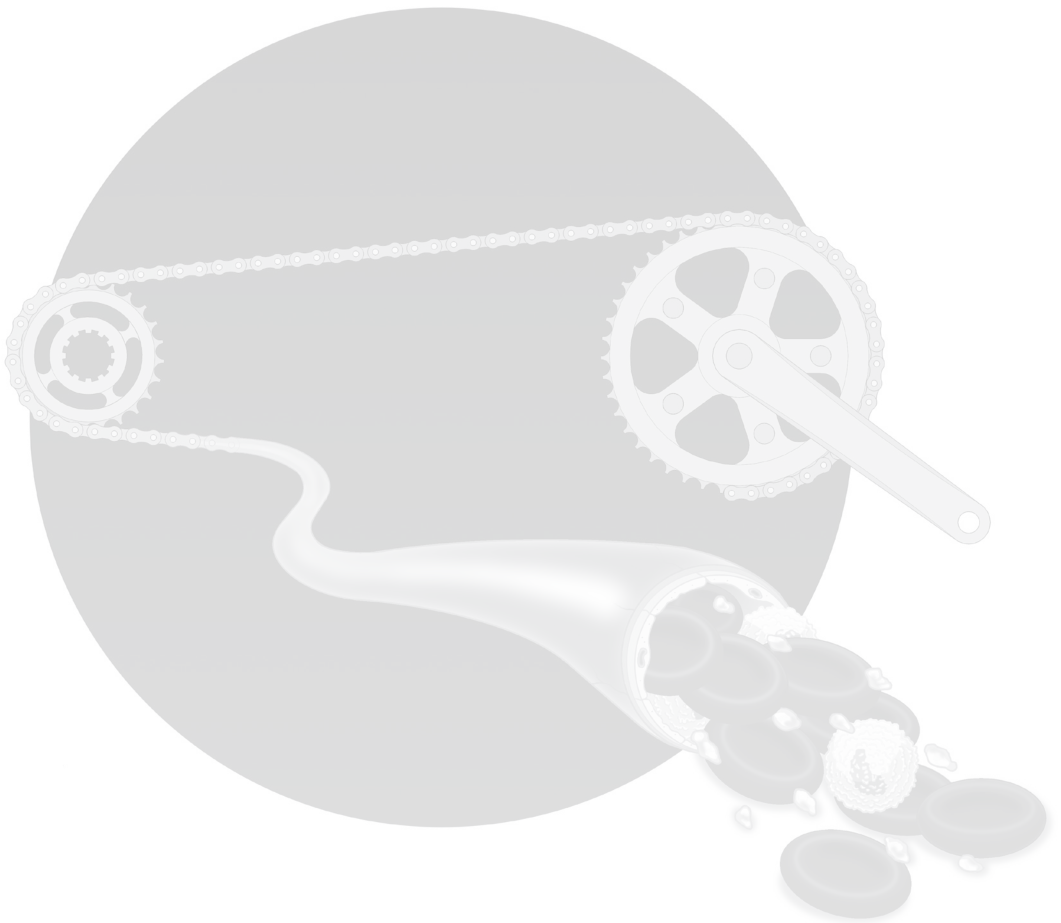

Chapter 6

Effect of storage time of platelet products on clinical outcomes after transfusion: a systematic review and meta-analyses

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Abstract

Background

Prolonged storage improves the availability of platelet products, but could also influence safety and efficacy. This systematic review and meta-analyses summarizes and quantifies the evidence of the effect of storage time of transfused platelets on clinical outcomes.

Methods

A systematic search in seven databases was performed up to February 2016. All studies reporting storage time of platelet products and clinical outcomes were included. To quantify heterogeneity, I^2 was calculated, and to assess publication bias, funnel plots were constructed.

Results

Twenty-three studies reported safety outcomes and fifteen efficacy outcomes. The relative risk of a transfusion reaction after old platelets compared to fresh platelets was 1.53 (95% confidence interval (CI): 1.04 to 2.25) (12 studies). This was 2.05 (CI 1.47 to 2.85) before and 1.05 (CI 0.60 to 1.84) after implementation of universal leukoreduction. The relative risk of bleeding was 1.13 (CI 0.97 to 1.32) for old platelets compared to fresh (5 studies). The transfusion interval was 0.25 days (CI: 0.13; 0.38) shorter after transfusion of old platelets (4 studies). Three studies reported use of platelet products, two for hematological patients, one for trauma patients. Selecting only studies in hematological patients, the difference was 4.51 units (CI1.92; 7.11).

Conclusion

Old platelets increase the risk of transfusion reactions in the setting of non-leukoreduction, shorten platelet transfusion intervals, thereby increasing the numbers of platelet transfusions in hematological patients, and may increase the risk of bleeding.

Introduction

Platelets are transfused to prevent or treat bleeding complications in patients with thrombocytopenia or platelet dysfunction.[1] Platelet products can be stored for a maximum of 4-7 days, depending on national guidelines and type of product.[2-5] During the period 2000-2002, a survey found the mean annual discard rate for 17 blood banks in 10 countries to be 13% (range 6.7-25%). As outdating was the main reason for discarding platelet products, prolonging storage is likely to reduce the number of discarded units.[6] However, *in vitro* studies demonstrated a gradual loss of platelet function during storage at room temperature, which is known as the 'storage lesion'.[7]
We previously performed a systematic review and meta-analyses on the effect of storage time at room temperature on clinical measurements. In these meta-analyses, older platelets had inferior results on all endpoints as compared to fresher products.[8] However, the clinical implications of these effects are not clear.[9,10] Therefore, the aim of the current systematic review and meta-analyses is to quantify the effect of storage time of platelet products on clinical outcomes after transfusion.

Methods

The search strategy, study selection, methods for assessing the risk of bias, and the data extraction were described previously and are in accordance with a pre-specified study protocol.[8]

<u>Search strategy</u>
In brief, a systematic search was applied to seven databases: MEDLINE (Pubmed), EMBASE, Cochrane, CINAHL, Academic Search Premier, ScienceDirect and Web of Science. Results were checked for missing relevant papers by experts in the field and the search strategy was adapted as needed. The search was last updated and performed in February 2016. The search strategy contained synonyms for platelets, fresh, old, and storage time. No limitations were placed on study design, language or year of publication (supplemental material).

Study selection

As specified in the study protocol, two reviewers independently screened titles and abstracts for relevance. Inclusion criteria were: performed in humans, concerning platelet transfusion, reporting clinical outcomes, reporting different storage times, and reporting original data. Disagreements were discussed with a third reviewer. The risk of bias was scored according to the 'Cochrane Collaboration's tool for assessing risk of bias' for randomized controlled trials[11] and 'Fowkes & Fulton tool' for randomized controlled trials and observational studies.[12] The items in the Fowkes & Fulton tool are appropriate study design, representative study sample, acceptable control group, quality of measurements and outcome, completeness, and confounding, which is similar as in the ACROBAT NRSI Cochrane tool for assessing non-randomized studies.[13] Papers scoring insufficient on one of these items were excluded.

Studies could only be included in the meta-analyses if they reported both a point estimate and a measure of precision. Further, studies needed to report an effect measure which could be recalculated to allow pooling with data from other studies (e.g. some studies reported only mean storage time in cases and controls, whereas risk ratios were reported in other studies). Papers written in other languages than English were translated and data extraction was verified by native speakers.

Data extraction

Storage time, type of outcome, product type, point estimate, and measure of precision were recorded. Authors of included studies were contacted when additional information was needed. If necessary, original results were recalculated in order to enable pooling of the results. In all cases where the underlying distribution could be assumed to be normal, mean and standard deviation were calculated from median, range and quartiles.[14] Results expressed in hours were recalculated to days.

Categorization

Storage time was dichotomized into fresh and old. Where storage time was already dichotomized, the reported dichotomization was maintained. Most papers defined fresh as ≤3 days and old as ≥4 days. Therefore these definitions were used to summarize results if papers reported multiple storage time categories, using standard formulas for combining samples sizes (Σn_i), means ($\sum \overline{x_i} * n_i/(\sum n_i)$) and standard deviations ($SD = (\Sigma(n_i - 1)s_i^2/sqrt[\Sigma(n_i - 1)]$) from multiple groups. Results were grouped by product: apheresis, pathogen-reduced apheresis (PR_aph), buffy coat in plasma (BC_plasma), buffy coat in platelet additive solution (BC_PAS),

pathogen reduced buffy coat in platelet additive solution (PR_BC PAS), and platelet rich plasma (PRP). If papers reported results concerning different products, these were handled as separate results.

Outcomes

Papers reporting laboratory measurements (i.e. corrected count increments, count increment, platelet recovery, survival, half-life) were reported elsewhere.[8]
Outcomes related to safety aspects were categorized into transfusion reactions, as defined by Delaney et al.;[15] complications, including other adverse events; mortality; and length of hospital stay. In-hospital mortality for trauma patients was assumed to be equivalent to 60 day mortality, if no additional data were available. In other words, we assumed that it was very unlikely that trauma patients who were discharged alive subsequently died within 60 days. The cut-off point of 60 days was chosen, as these data were available in other papers reporting mortality.
Outcomes related to efficacy aspects were categorized into bleeding; transfusion interval; transfusion need (i.e. number of platelet, red blood cell, and plasma transfusions, or amount of cryoprecipitate during hospital stay or period of five days, as reported); repeated transfusion within 24 hours; and hemostatic potential as measured by thromboelastography.

Statistical analyses

For studies reporting only incidences of transfusion reactions, complications, mortality, and bleeding, the relative risk was calculated using standard formulas.[16] The corresponding 95% confidence intervals were calculated using Fisher's exact test. Standard errors were determined from the confidence intervals. For case control studies, odds ratios were calculated with standard errors according to the formula of Woolf.[17] The included case control studies selected controls in a way which allowed the reported odds ratios to be interpreted as relative risks.[18] These odds ratios were therefore treated as relative risks in all analyses. Relative risks reflecting the risk of stoppage of bleeding, or improvement in bleeding rate were recalculated to reflect the risk of no stopping of bleeding or no improvement of bleeding rate.
For continuous outcomes, weighted mean differences (WMD) were calculated. If more than ten studies were included, a pre-specified subgroup analysis was performed, based on product type (i.e. before or after implementation of universal leukoreduction). Metaregression was performed to examine the impact of product type on the pooled estimate. The adjusted R-squared ($R_{adj}^2 = (\hat{\tau}_0^2 - \hat{\tau}^2)/\hat{\tau}_0^2$) was calculated to examine the proportion of heterogeneity explained by product type. A

sensitivity analysis was performed, excluding the studies with the largest standard errors and meeting abstracts.

To assess the risk of publication bias, funnel plots were generated and Egger's bias coefficient was calculated.[19] A single funnel plot was made for all continuous endpoints combined. To standardize all outcomes to the same scale, the standardized mean difference (SMD) was calculated for each comparison. The standardized mean difference expresses the size of the intervention effect in each comparison relative to the standard deviation estimated in that comparison.[20] All studies were centered around the point of no effect by subtracting the pooled standardized mean difference for each outcome from the standardized mean difference for that outcome of each comparison.

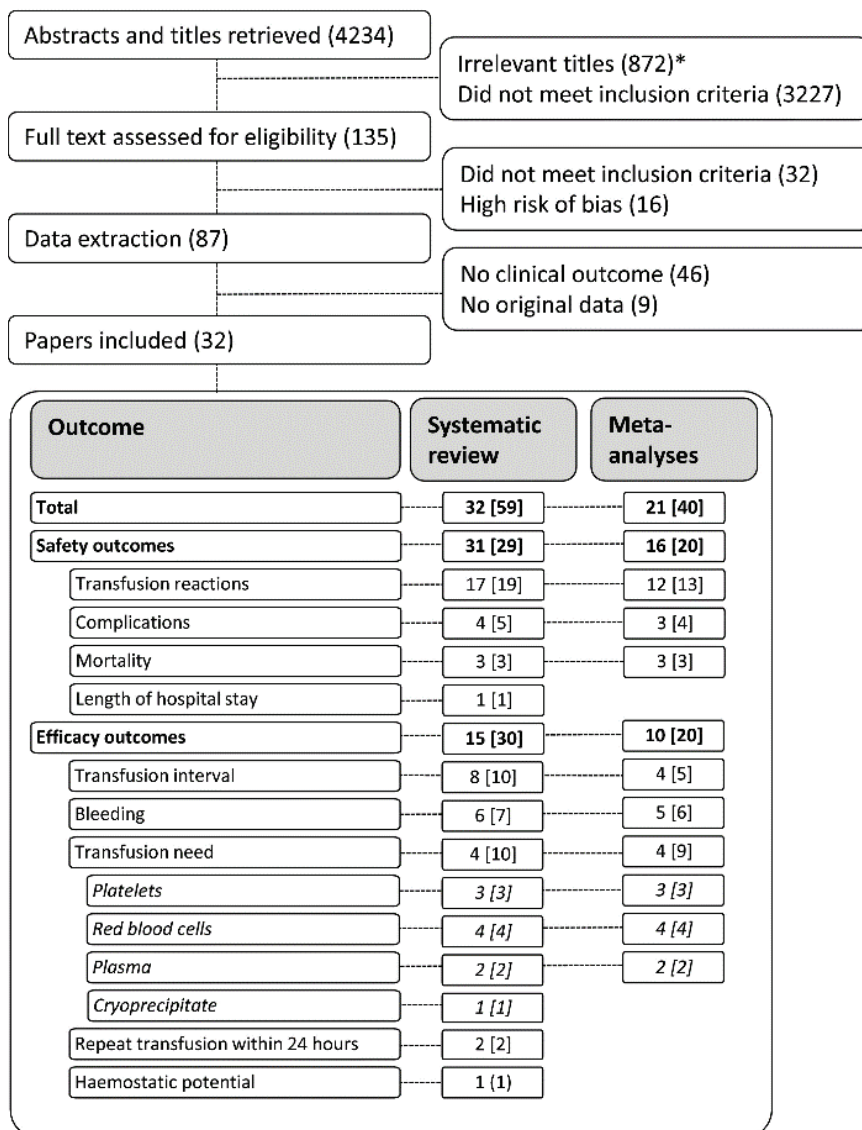
Heterogeneity was quantified by the I^2 statistic.[21] To account for substantial heterogeneity a random effects model was used for all meta-analyses. As a sensitivity analysis, we performed a meta-analysis including only the observational studies. All statistical analyses were performed using Stata version 14, packages metan and metareg.

Results

Selection

The literature search yielded 4,234 papers, of which title and abstract were screened for the predefined inclusion criteria, as described previously.[8] Following selection on inclusion criteria and the risk of bias, 32 studies, reporting 59 unique comparisons, were included in this systematic review (figure 1). This included five meeting abstracts and 27 original papers. Four papers reported on trials in which storage time was randomized. Twenty-three studies reported on observational cohort studies, of which five were secondary analyses on data of randomized trials. Five papers reported on case control studies. Thirty-one papers were written in English and one in Chinese. Included studies are described in more detail in the supplemental material, table S1.

Figure 1. Flow chart of study selection



Numbers represent numbers of papers. Some papers reported comparisons for more than one outcome or multiple comparisons for a single outcome. Numbers in square brackets represent the number of unique comparisons.

Table 1. Description of studies retrieved by the literature search, but not reporting data necessary for pooling in the meta-analyses.

Author and year	Product[a]	Definition fresh	Favours old	No difference	Favours fresh	Definition old	Group size fresh vs. old [*N* transfusions][b]	Outcome (fresh vs. old or controls vs. cases)
Transfusion reactions								
Heddle 1993	PRP or Aph	1–3			■	4–5	Total 65 transfusions	Slope logistic regression $P = 0{,}004$[c]
Lane 1997	Aph	3·1		■		3·8	36 controls vs. 9 cases	3·1 ± 1·1 vs. 3·8 ± 0·7 days[d]
Patterson 2000	PRP_nonL	≤3			■	>3	338 vs. 789 transfusions	Slope linear regression 0·0305; $P < 0{\cdot}001$[c]
Silliman 2003	PRP or Aph	4·2			■	4·5	225 controls vs. 46 cases	4·2 ± 0·1 vs. 4·5 ± 0·2 days, $P = 0{\cdot}014$[d]
Patterson 2000	PRP	≤3		■		>3	306 vs. 1023 transfusions	Slope linear regression 0·009; $P = 0{\cdot}5$[c]
Liu 2013	Aph	2·87		■		2·92	13 controls vs. 16 cases	2·87 ± 0·82 days vs. 2·92 ± 1·03[d]
Complications								
Vande Vusse 2014	PRP or Aph			■			906 patients, 75 cases	HR: 0·84 (CI: 0·51–1·37)[c]
Length of ICU stay								
Inaba 2011	Aph	≤3		■		4–5	128 [205] vs. 253 [380] patients	Median 6 (range 1–181) vs. 6 (1–181) days
Shorter interval between transfusions								
Norol 1994	Aph	≤8 h			■	≤2	141 [141] vs. 141 [141] patients	3·1 vs. 2·3 days
Benjamin 2003	Aph	1–2		■		4–5	697 vs. 1247 transfusions	2·0 vs. 2·0 days; $P = 0{\cdot}97$
Benjamin 2003	PR_aph	1–2	■			4–5	383 vs. 1176 transfusions	1·4 vs. 1·6 days; $P = 0{\cdot}18$
Heuft 2013	Aph	1–4			■	1–5	36 [191] vs. 41 [250] patients	2·0 vs. 1·1 days; $P < 0{,}001$
Slichter 2005	PRP or Aph	<2			■	3–5	Total 5423 transfusions in 525 patients	Difference 0·19 days (CI: 0·12–0·26)
Time to first who ≥2 bleeding								
Triulzi 2012	BC or Aph	3		■		5	156 vs. 217 patients	HR: 1·02 (CI: 0·62–1·70)
Transfusion need: cryoprecipitate								
Inaba 2011	Aph	≤3		■		4–5	128 vs. 253 patients	Median 0 (range 0–33) vs. 0 (0–22) units
Repeated transfusion ≤24 h								
Norol 1994	Aph	≤8 h			■	≤2	88 vs. 88 transfusions	RR: 6·2 (CI: 2·5–15·4)
Duguid 1991	BC_plasma	1–2			■	3–5	77 vs. 40 transfusions	RR: 2·3 (CI: 1·1–4·8)
Haemostatic potential (TEG)[e]								
Roeloffzen 2010	BC_plasma	1–3			■	4–5	35 vs. 35 patients	*K*-time: 27 ± 16 vs. 37 ± 22 min $P = 0{\cdot}03$[e] Alpha angle: 13° ±10° vs. 8°±8° $P = 0{\cdot}02$

Studies can appear more than once if multiple products or multiple end-points were reported.

Studies were excluded from the meta-analyses if no measure of precision was reported or if effect measure could not be recalculated in order to allow pooling of results.

■ Conclusion of the paper. 'No difference' means paper found no relevant differences between the groups.

[a]Product codes: aph, apheresis; BC_plasma, buffy coat stored in plasma; PRP, platelet-rich plasma, nonL, non-leucoreduced; PR, pathogen reduced.

[b]Group size is expressed as number of patients [number transfusions], unless otherwise specified.

[c]Storage time analysed as categorical variable, per day.

[d]Storage time in controls vs. storage time in cases.

[e]Thromboelastography measurements: *K*-time: time until a fixed level of clot firmness is reached in minutes, Alpha angle: rate of clot growth in degrees.

Safety outcomes

Transfusion reactions

One randomized trial, two secondary analyses of randomized trials, nine cohort studies and five case control studies reported transfusion reactions (figure 1). In ten papers different kind of transfusion reactions were reported as one combined endpoint. In three papers transfusion reactions were specified as febrile non-hemolytic transfusion reactions, in two papers as transfusion related acute lung injury (TRALI), in one paper as allergic transfusion reactions, and in one paper as septic transfusion reactions.

Twelve studies (thirteen comparisons) were included in the meta-analysis. The pooled risk ratio of old versus fresh platelets was 1.53 (95% confidence interval (CI): 1.04 to 2.25, I^2 83.1%) (figure 2). Before universal leukoreduction was introduced this risk ratio was 2.05 (CI: 1.47 to 2.85, I2 55.6%) and after introduction it was 1.05 (CI 0.60 to 1.84, I2 80.8%). The relative risk ratio of leukoreduced products compared to non-leukoreduced products was 0.51 (CI: 0.31 to 0.86, I2 68.1%). Adjustment for leukoreduction explained 42.36% of heterogeneity. Eggers bias coefficient was 1.62 (p=0.26) (supplemental material). Selection of the observational studies yielded a relative risk of 1.05 (CI 0.57 to 1.92) (supplemental material). This was similar to the risk ratio in the randomized trial (RR 1.10, CI 0.22 to 5.40). An additional analysis excluding the meeting abstracts and smaller studies, gave similar results (supplemental material). Five studies (six comparisons) were excluded from the meta-analysis. Three were case control studies comparing mean storage time in both groups, one study did not report the group sizes, and one (two comparisons) only reported a regression coefficient. Of these six comparisons, two reported no difference in incidence of transfusion reactions between both storage time categories in leukoreduced products, three reported an increased incidence after exposure to older non-leukoreduced platelets, and one reported no difference of mean storage time in cases and controls who received leukoreduced as well as non-leukoreduced products (table 1).

Other safety outcomes

Four cohort studies reported complications. Reported complications were: major infections, defined as pneumonia, positive blood culture, leg wound infection, sternal wound infection, or mediastinitis; positive blood culture; idiopathic pneumonia syndrome; and a composite endpoint of sepsis, ARDS, renal failure, or liver failure. Three studies, four comparisons, were included in the meta-analysis. The pooled risk ratio for these complications of old versus fresh platelets was 1.07 (CI: 0.83; 1.38, I^2 66.6%) (figure 2). One paper could not be included in the meta-

analysis, as it reported a hazard ratio of risk of idiopathic pneumonia syndrome, which was 0.84 (CI 0.51 to 1.37).

One randomized trial and two cohort studies reported mortality.[22-24] All were included in the meta-analysis. The pooled risk ratio for mortality was 1.03, (CI: 0.86 to 1.24, I^2 0.0%) (figure 2). The pooled risk ratio in observational studies was 1.03 (CI 0.86 to 1.25) compared to 0.93 (CI 0.29 to 2.96) in the randomized trial was (supplemental material).

Length of ICU stay was reported by one study, which found no difference for trauma patients receiving fresh or old platelets.

Figure 2. Forest plot safety outcomes and platelet storage time

Panel A. Meta-analyses of transfusion reactions and platelet storage time, stratified by implementation of universal leukoreduction.

Panel B. Meta-analyses of complications and mortality and platelet storage time.

The numbers represent the relative risk of old platelets compared to fresh platelets with corresponding 95% confidence interval for each study.

** Product codes: Aph = apheresis, , PRP = platelet rich plasma, BC-PAS = buffy coat stored in PAS, BC-plasma = buffy coat stored in plasma PR = pathogen-reduced.*

† FNHTR = Febrile non haemolytic transfusion reaction.

‡ TRALI = Transfusion related acute lung injur

Efficacy outcomes

Transfusion interval

Three randomized trials, two secondary analyses of randomized trials and three cohort studies reported a transfusion interval. Four studies (five comparisons) were included in the meta-analysis. The interval between transfusions was 0.25 days (CI: 0.13 to 0.38, I^2 19.5%) longer after transfusion of fresh platelets (figure 3). The weighted mean difference in the observational studies was 0.19 days (CI 0.14 to 0.25) and in the two randomized trials it was 0.42 days (CI 0.10 to 0.75) (supplemental material). Four papers (five comparisons) were excluded from the pooled analysis, as these did not provide the necessary measure of precision. Three reported a longer interval following transfusion of fresh platelets. One paper reported no difference in interval following transfusion of apheresis platelet products and a shortened interval after transfusion of fresh pathogen reduced products (table 1). Using the number of transfusions per study as weighing factor, the mean interval reported by the papers excluded from the meta-analysis was 0.14 days.

Bleeding

Two randomized trials, two secondary analyses of randomized trials and two cohort studies reported data about bleeding. Reported bleeding endpoints were: incidence of any bleeding symptoms; incidence of bleeding in the central nervous system; percentage of transfusions resulting in lower WHO grade of bleeding; incidence of stopping of gastrointestinal bleeding, hemorrhagic cystitis or epistaxis; proportion of days with bleeding as measured by daily monitoring; and time from transfusion to first WHO grade 2 bleeding. In four studies patients were assessed for bleeding symptoms daily. In two studies medical records were reviewed for bleeding symptoms. Five studies (six comparisons) were included in the meta-analysis. The pooled risk ratio of old platelets versus fresh platelets for any bleeding symptom was 1.13 (CI: 0.97 to 1.32, I^2 38.4%). The pooled risk ratio in observational studies was 1.18 (CI 0.99 to 1.41) and in the two randomized trials the pooled risk ratio was 0.86 (CI 0.58 to 1.27) (supplemental material). Exclusion of the meeting abstracts gave similar results (supplemental material). One paper could not be included in the pooled analysis, as it reported the time to first ≥WHO grade 2 bleeding (hazard ratio old versus fresh: 1.02 CI: 0.62 to 1.70).

Transfusion need

One randomized trial and three cohort studies reported the need of transfusions. This was reported during hospital stay or during a period of five days. Three papers

(three comparisons) were included in the meta-analysis on need of platelet transfusion. The weighted mean difference was 2.76 fewer products (95% CI: -1.11 to 6.64, I^2 84.1%) with fresh platelets compared to old platelets (figure 3). Two studies were performed among hematological patients and one among trauma patients. Selecting only studies in hematological patients yields a weighted mean difference of 4.51 units (CI 1.92; 7.11). The weighted mean difference in the two observational studies was 1.66 units (CI -2.32 to 5.64), and in the randomized trial it was 6.00 units (CI 0.90 to 11.10) (supplemental material).

Four papers (four comparisons) were included in the meta-analysis on need of red blood cell transfusions. The weighted mean difference was 0.08 products fewer (95% CI: -0.18 to 0.34, I^2 3.2%) after transfusion of fresh platelets. The weighted mean difference in the observational studies was 0.07 units (CI -0.06 to 0.25), and this was 2.50 units (CI -1.23 to 6.23) in the randomized trial (supplemental material).

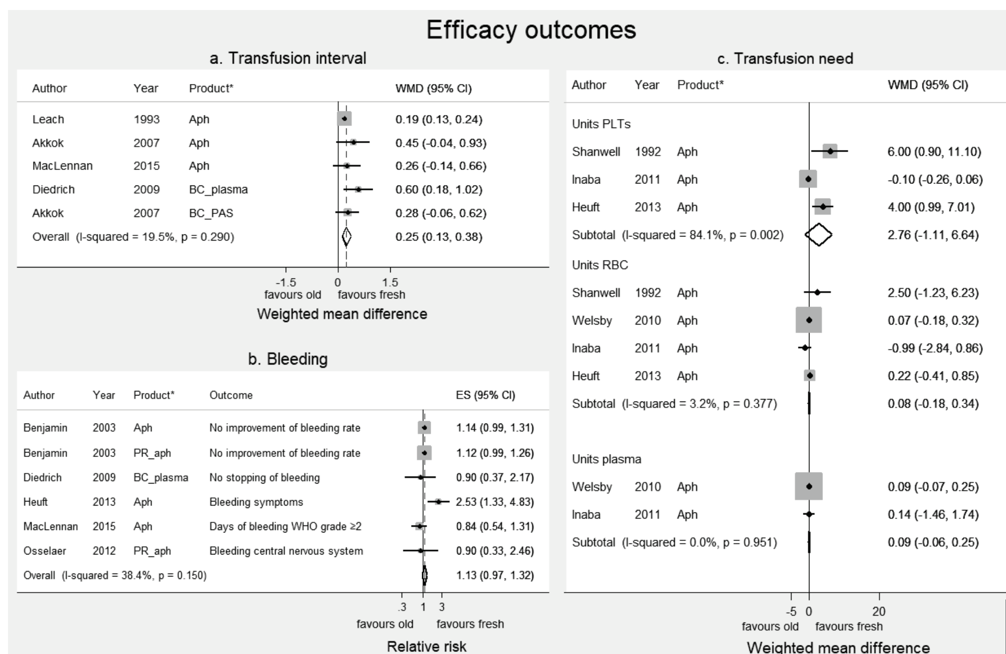
Two papers (two comparisons) were included in the meta-analysis of need of plasma transfusions. The weighted mean difference was 0.09 products fewer (95% CI: -0.06 to 0.25, I^2 0.0%) after transfusion of fresh platelets (figure 3). One study reported the need of cryoprecipitate, which was not different after transfusion of fresh or old platelets (table 1).

Other efficacy outcomes

One randomized trial and one cohort study reported an increased risk of a repeated transfusion within 24 hours (table 1). Results from these studies could not be pooled as the storage time of the old platelets in one paper coincided with the storage time of the fresh platelets in the other.

One study determined the hemostatic potential of platelets using thromboelastography (TEG) and reported better hemostatic properties of fresh platelets compared to old platelets (table 1).

Figure 3. Forest plot of studies reporting efficacy outcomes and storage time



A. Forest plot of studies comparing the interval between subsequent platelet transfusion in days. The numbers represent the weighted mean difference (WMD), calculated as: 'interval fresh' – 'interval old'.
B. Forest plot of studies reporting the risk of bleeding. The numbers represent the relative risk of old platelets compared to fresh platelets with corresponding 95% confidence interval for each study.
C. Forest plot of studies reporting transfusion need. The numbers represent the weighted mean difference, calculated as 'number of products old' – 'number of products fresh'.
** Product codes: Aph = apheresis, BC-PAS = buffy coat stored in PAS, BC-plasma = buffy coat stored in plasma, PR = pathogen-reduced.*
† Results shown for all studies. Selecting only studies in hematological patients yields a weighted mean difference of 4.51 units (CI 1.92; 7.11).

Discussion

To conclude, transfusion of older platelet products was associated with more transfusion reactions before the implementation of universal leukoreduction. This association disappeared after the implementation of universal prestorage leukoreduction. Transfusion of older platelet products was associated with a shorter time to the next transfusion, a trend towards a higher risk of bleeding, and in hematogical patients an increased need of platelet transfusions. Storage time of

platelet concentrates was not associated with the risk of mortality nor the consumption of other blood products.

The association between storage time and laboratory measurements (i.e. platelet counts and derivatives thereof) has been reported elsewhere. That study reported inferior results for older platelets for all relevant measurements.[8] The current results suggest that these lower laboratory values are associated with a higher risk of bleeding and a shorter time to the next transfusion. Decreased efficacy of old platelets could explain the increased bleeding risk. Another explanation could be that platelet count is routinely measured on fixed moments, e.g. three times a week. Transfusion of older platelets results in lower increments, leading to a lower platelet count on average in case of a prophylactic transfusion strategy. This could result in an increased bleeding risk.

The increased risk of transfusion reactions in old platelets could be attributed completely to studies performed before the implementation of pre-storage leukoreduction. Leukocytes and leukocyte-derived cytokines are thought to be a major cause of febrile non hemolytic transfusion reactions.[25,26]

With the implementation of universal leukoreduction an absolute risk reduction of 25.1% was expected in the risk of febrile non hemolytic transfusion reactions.[27] The results of the present meta-analyses confirm the beneficial effect of pre-storage leukoreduction on the incidence of transfusion reactions.

An important strength of these meta-analyses is that we were able to pool the available data on bleeding risk. Most studies are powered to study other outcomes and are therefore by themselves inconclusive on bleeding risk. Although different definitions of bleeding are used, we assume storage time has the same effect on all symptoms and it is appropriate to pool the estimates.

Another strength of this study is the broad search strategy. No limits were used for study design, year or language. Therefore, a maximum of available papers reporting clinical effects of storage time have been retrieved and all reported clinical outcomes were studied.

The broad search strategy also returned meeting abstracts, which are possibly more prone to bias. Exclusion of the meeting abstracts did not change the results of the main analyses, indicating these abstracts estimate the same effect. Due to the limited number of randomized trials it was not feasible to perform a sensitivity analysis including only randomized trials. However, the pooled estimates of the observational studies were comparable with the results of the randomized trials. This suggests that the observational studies are reliable, allowing inclusion in the

meta-analysis. The relatively large difference between the estimates of the observational studies and the randomized trials in transfusion interval is based on one precise observational study in which the difference in interval was 0.19 days (CI 0.13 to 0.24).

The main limitation of this study is that storage time had to be dichotomized into two broadly defined categories, fresh and old. Most studies reported differences between two groups and defined fresh as storage time of ≤3 days. Therefore it was impossible to compare the safety and efficacy of platelets stored for 1-5 days with platelets stored for 6-7 days. Whereas this is the difference between storage duration used in the Netherlands, compared with several other countries.[2-5]
Not all retrieved studies could be included in the meta-analyses, which could potentially induce selection bias. However, the studies excluded from the meta-analysis regarding transfusion interval, reported on average a similar interval as the pooled estimate of the meta-analysis and for the outcomes transfusion reactions and bleeding, the results of the excluded studies pointed in the same direction.

Another limitation of this study is the large heterogeneity between studies reporting transfusion reactions (I^2 83.1%). This is partly due to the difference in effect observed before and after the implementation of universal leukoreduction. Correction for leukoreduction in metaregression explained 42% of this heterogeneity. Other sources of variation could include the lack of standardized definitions and differences between active and passive monitoring of transfusion reactions. Among studies reporting bleeding symptoms heterogeneity was moderate. This could be due to the fact that several different definitions of bleeding are used and it is measured in different ways. The number of studies reporting on the other outcomes was smaller and therefore it is difficult to detect heterogeneity and publication bias for these outcomes.

In conclusion, the safety and efficacy of platelet products deteriorates during storage. However, leukoreduction reduces the risk of transfusion reactions following transfusion of old platelets effectively. Efficacy of platelet transfusions is reduced after prolonged storage, leading to a shorter interval to the next platelet transfusion. Transfusion of old platelet concentrates might increase the risk of bleeding.

Supplemental material

Available at
http://onlinelibrary.wiley.com/doi/10.1111/vox.12494/abstract#footer-support-info

Data S1. Search queries.
Table S1. Overview of all included comparisons per product.
Figure S1. Funnel plot of studies comparing incidences of transfusion reactions.
Figure S2. Funnel plot of efficacy of platelet transfusion.
Figure S3. Sensitivity analysis: forest plot transfusion reaction, bleeding and platelet storage time.
Figure S4.Sensitivity analysis: forest plot safety outcomes, excluding the randomized trials.
Figure S5. Sensitivity analysis: forest plot efficacy outcomes, excluding the randomized trials.
Figure S6. Forest plot transfusion interval and transfusion need and platelet storage time, standardized analysis.

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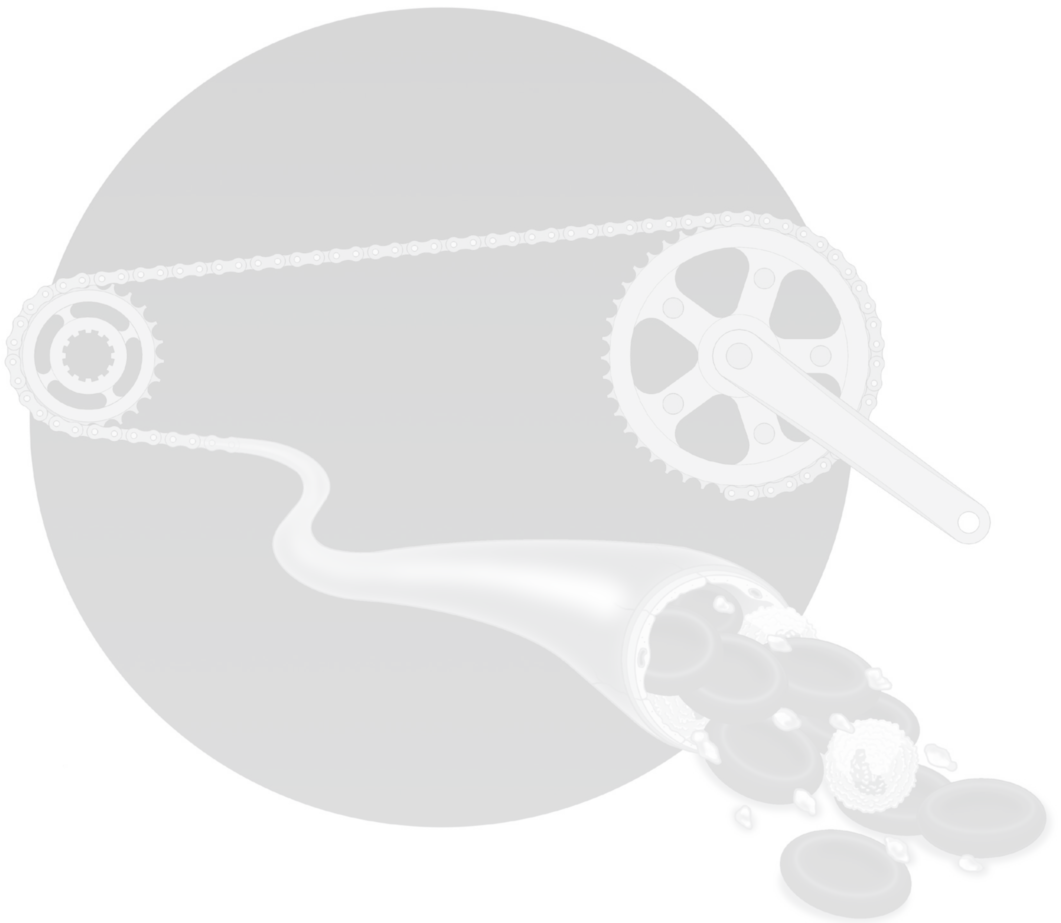

Chapter 7

Storage time of platelet concentrates and all-cause bacteremia in hematological patients

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Abstract

Background
Extension of storage time of platelet concentrates may result in an increased risk of bacteremia, directly via transfusion of contaminated products or indirectly via transfusion related immunomodulation. We aimed to quantify the association of storage time of platelet concentrates and all-cause bacteremia in hematological patients.

Design and methods
We established a cohort of hematological patients who received a platelet transfusion between 2005 and 2015. Cases were defined as patients with a bacteremia the day after transfusion, and matched to as many controls as possible. A conditional logistic regression was performed, stratified by storage medium.

Results
Among 3,514 patients receiving 36,032 platelet concentrates stored in plasma, 613 cases of bacteremia were found. The relative risk of all-cause bacteremia the day after transfusion was 0.80 (CI 0.58-1.12) for platelet concentrates stored 3-4 days and 0.67 (CI 0.49-0.92) for ≥ 5 days, compared to ≤2 days. Among 1,527 patients receiving 11,822 platelet concentrates stored in platelet additive solution (PAS), 182 cases of bacteremia were found. The relative risk of all-cause bacteremia was 1.14 (CI 0.70-1.84) for platelet concentrates stored 3-4 days and 1.19 (95% CI 0.70-2.01) for ≥ 5 days, compared to ≤2 days.

Conclusion
Storage time of platelet concentrates was not associated with increased occurrence of all-cause bacteremia the day after transfusion. If anything, fewer bacteremia occurred with increasing storage time of platelet concentrates in plasma. These bacteremias are not directly caused by transfusion of a contaminated product and the underlying mechanism warrants further research.

Introduction

Transfusion of platelets is an important aspect of supportive care in the treatment of patients with hematological malignancies, to prevent or treat bleeding complications during periods of severe thrombocytopenia.[1,2] The concurrent neutropenia predisposes these patients to infectious complications.[3]

Transfusions can directly cause bacterial infections via transmission of bacteria through contaminated products. In particular platelet concentrates may carry this risk, as these are stored at room temperature, allowing bacterial proliferation. This is clearly illustrated by several case reports of severe bacterial sepsis after transfusion of contaminated platelet concentrates.[4-7] In an attempt to reduce this risk, storage time is limited to 3.5 days in Japan and to four and five days in the USA and Germany. [8-11] A large trial in the USA, which aimed to investigate the safety of seven days storage with the implementation of early testing, was terminated early due to concerns about the residual risk of transfusion of a contaminated platelet concentrate.[12] However, storage up to seven days in combination with bacterial screening is allowed in, among others, Spain, the United Kingdom, Sweden and the Netherlands.[11] Bacterial screening does not eliminate the risk of septic reactions completely as false negative results occur. In most studies, septic reactions were associated with platelet concentrates stored for four to six days.[5,13-16]

The risk of infections does not solely depend on sterility of the platelet concentrate. Besides direct transmission of infections with a contaminated product, it has been speculated that platelets itself play a role in the immune response and that transfusions could modulate this response.[17-19] Immunosuppressive effects of a transfusion could result in an increased incidence of all-cause bacteremias.

The aim of this study was therefore to quantify the association of storage time of platelet concentrates screened for bacterial contamination and stored for up to seven days in plasma or platelet additive solution (PAS) with all-cause bacteremia in a large cohort of hematological patients.

Methods

Design and population

We performed a case control study, nested in a cohort of recipients of platelet transfusions from nine hospitals in the Netherlands, three university and six general hospitals (supplemental material, table S1). The study population consisted of all patients with a hematological malignancy or aplastic anemia who had received at least one platelet transfusion between January 2005 and December 2015. The study period varied between participating hospitals (supplemental material). Patients were selected based on DBC code (Diagnosis treatment combination). Selected diagnoses were leukemia, lymphoma, myeloma, and aplastic anemia (selected codes are depicted in the supplemental material). We excluded patients younger than one year, as transfusion policies in neonates differ from those of the general population. The study protocol was approved by the Medical Ethical Committee of each participating hospital.

Platelet products

Buffy-coats were produced from whole blood after overnight hold and leuko- and plasma-reduced. Buffy-coats of five donors were pooled and re-suspended in plasma or platelet additive solution (PAS), with 25 mL of plasma per donor to a final volume of 300-350ml.[1,20] The geographic location of the hospital determined which storage medium was used.[21] Transfusion of platelet concentrates stored in storage medium not normally used in that hospital were assumed to be given for exceptional indications and therefore excluded from all analyses. PAS-B (T-sol, Baxter) was used as storage medium through 2012, with PAS-C (Intersol, Fenwal, Inc) being used as of January 2013. Maximum storage time for platelets stored in PAS-B was five days. Platelets stored in PAS-C or plasma could be stored for a maximum of seven days. Hyper-concentrated products and platelet concentrates collected via apheresis were excluded from all analyses as these were only used for specific indications.[1] All platelet concentrates were sampled immediately after preparation and screened for bacterial contamination with the BacT/Alert system consisting of an aeroob and anaeroob culture bottle, inoculated with 7.5ml each, and released on a ‘negative-to-date’ basis.[1,20]

Variables

Characteristics of blood products were extracted from the national blood bank system. Recorded variables were donation date, storage medium, ABO and RhD blood group, and product type. Storage time was counted in days from the day of donation (day 0) up to and including the day of transfusion. Storage time was categorized into three groups: ≤2 days, 3-4 days, ≥5 days. Product identification numbers were used to link this information to clinical data. Patient characteristics were extracted from the electronic health care system of the participating hospitals. Recorded variables were age, gender, AB0 and RhD blood group, positive blood cultures, transfusions of platelets, and all DBC codes.

Cases

Cases were defined as patients who received at least one platelet transfusion and had a bacteremia the day after transfusion. In order to select these cases, we linked clinical data, including all positive blood cultures, to transfusion data using the patient identification numbers. If a patient received multiple transfusions of different storage time categories on the same day, these transfusion-days were excluded from all analyses. A bacteremia was defined as a positive blood culture. Blood cultures were not standardly performed the day after transfusions, but only taken on indication or scheduled in certain treatment protocols. One patient could develop multiple bacteremias. A period of fourteen days between two positive blood cultures, regardless of negative cultures in between, was required to ensure two bacteremia episodes were unrelated.

Controls

Cases were matched to as many control transfusion-days as possible. If a case received platelet transfusions on several days, all transfusions which were not followed by a positive blood culture could be included as control for this or other cases (i.e. one patient could be included as case as well as control). Matching factors were hospital, day of the week, number of transfusions on a single day, AB0 blood group, and storage medium. To account for this matching, a conditional logistic regression was performed using the youngest storage time category as a control for the exposure and adjusted for the matching factors. As the controls derive from the entire cohort, the odds ratios could be interpreted as relative risks.[22,23]

Additional analyses

We performed five additional analyses to explore the impact of possible sources of bias and effect modification.

First, we performed a subgroup analysis among patients with the highest risk of infections. Here we limited the analysis to intensively treated hematological patients by selecting patients with a diagnosis of acute leukemia, or high grade non-Hodgkin lymphoma.

Second, we investigated the association of storage time in different generations of additive solutions, i.e. PAS-B and PAS-C.

Third, we investigated the association of different generations of platelet additive solutions with bacteremia, stratified by storage time category. This was possible as prior to 2013 the Dutch blood supply organization used exclusively PAS-B as an additive solution, whereas after 2013 exclusively PAS-C was used. Therefore we used calendar time as instrumental variable in this analysis.

Fourth, we used a negative control to explore any residual confounding.[24] Therefore, we selected cases with bacteremia the day before transfusion.

Fifth, to explore any immune-modulatory effects of storage, we investigated the association of bacteremia with storage time of platelet concentrates transfused two or three days before. Patients who received transfusions on several days before bacteremia were excluded from this analysis (i.e. in the analysis regarding transfusions given three days before bacteremia, we excluded patients who also received a transfusion one or two days before bacteremia).

Results

Study population

The total cohort consisted of 5,008 patients who received 47,854 platelet transfusions on 43,450 days (figure 1 supplemental material). Patients were on average 56.5 years old (SD 17.8), 60.8% of patients were male, and 43.8% were diagnosed with acute leukemia. On 62.9% of analyzed days a plasma stored platelet concentrate was given to a patient with acute leukemia, which was on 56.3% of days for platelets stored in PAS (table 1). Patient received one transfusion (range 1 to 10 transfusions) on 91.4% of the analyzed days. 660 patients developed bacteremia the day after transfusion, for a total of 795 transfusion-days, with a median of 1 (range 1 to 6) bacteremia per patient.

Median storage time of platelet concentrates stored in plasma was 5 days (interquartile range [IQR]: 4 to 6 days) and 4 days (IQR: 3 to 5 days) for platelet

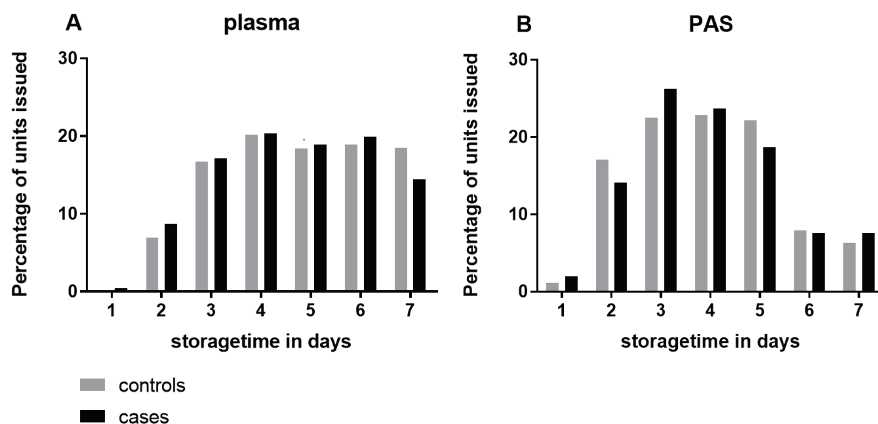
concentrates stored in PAS. Median storage time was 3 days (IQR: 3 to 4 days) for platelet concentrates stored in PAS-B and 5 days (IQR: 3 to 6 days) for platelet concentrates stored in PAS-C. The distribution of storage time for cases and controls, stratified by storage medium, is shown in figure 1 and in the supplemental material for the different generations of PAS.

Table 1. Baseline characteristics patients

Plasma	**Total**	**≤2 days**	**3-4 days**	**≥5 days**
Transfusion days	32,734	2,390 (7.3)	12,100 (37.0)	18,244 (55.7)
Patients*	3,514	1,240	2,671	3,030
Age in years, mean (SD)	52.8 (17.6)	52.3 (17.3)	52.6 (17.5)	53.1 (18.0)
Male sex (%)	20,856 (63.7)	1,540 (64.4)	7,672 (63.4)	11,644 (63.8)
Number of transfusions per day, median (range)	1 (1-10)	1 (1-6)	1 (1-10)	1 (1-8)
Diagnosis				
-Acute leukemia	20,575 (62.9)	1,409 (58.9)	7,596 (62.8)	11,570 (63.4)
-Lymphoma	4,955 (15.1)	413 (17.3)	1,802 (14.9)	2,740 (15.0)
-Myeloma	2,275 (6.9)	160 (6.7)	871 (7.2)	1,244 (6.8)
-Chronic leukemia	2,289 (7.0)	188 (7.9)	846 (7.0)	1,255 (6.9)
-Aplastic anemia and other	2,640 (8.1)	220 (9.2)	985 (8.1)	1,435 (7,9)
PAS	**Total**	**≤2 days**	**3-4 days**	**≥5 days**
Transfusion days	10,716	1,994 (18.6)	4,840 (45.2)	3,882 (36.2)
Patients*	1,527	798	1,180	1,051
Age in years, mean (SD)	59.4 (15.0)	58.6 (14.7)	59.5 (15.1)	59.9 (15.0)
Male sex	6,633 (61.9)	1,174 (58.9)	3,000 (62.0)	2,459 (63.3)
Number of transfusions per day, median (range)	1 (1-5)	1 (1-5)	1 (1-5)	1 (1-4)
Diagnosis				
-Acute leukemia	6,033 (56.3)	1,053 (52.8)	2,692 (55.6)	2,288 (58.9)
-Lymphoma	2,192 (20.5)	457 (22.9)	1,003 (20.7)	732 (18.9)
-Myeloma	1,258 (11.7)	271 (13.6)	579 (12.0)	408 (10.5)
-Chronic leukemia	577 (5.4)	133 (6.7)	260 (5.4)	184 (4.7)
-Aplastic anemia and other	656 (6.1)	80 (4.0)	306 (6.3)	270 (7.0)

Numbers represent number of transfusion days (percentages) unless otherwise specified.
** Total numbers reflect unique patients. Numbers in subgroups don't add up till total numbers, since one patient could contribute transfusion-days to several storage time categories.*

Figure 1. Storage time of platelet concentrates for cases and controls

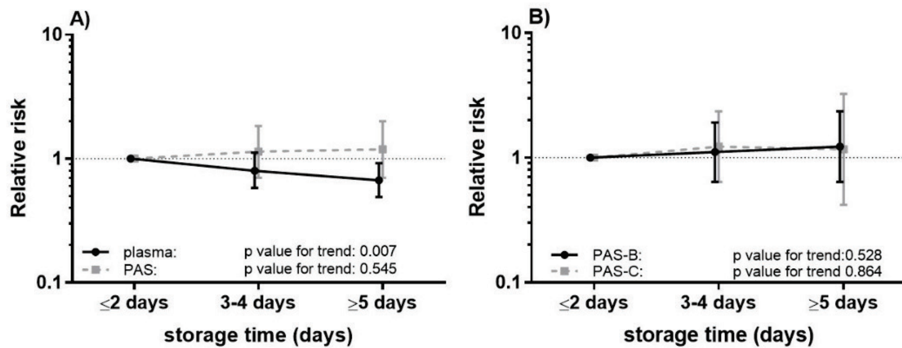


Panel A) storage time of platelet concentrates stored in plasma
Panel B) storage time of platelet concentrates stored in PAS

<u>Platelets in plasma</u>

Among 3,514 patients receiving 36,032 plasma-stored platelet concentrates on 32,734 different days, 613 cases of bacteremia were detected the day after transfusion. In 56 cases the patient had received a platelet concentrate stored for ≤2 days (incidence 2.34/100 transfusion-days; 95% confidence interval (CI): 1.76 to 3.04), in 232 cases a platelet concentrate stored for 3-4 days (incidence 1.91/100 transfusion-days; CI: 1.68 to 2.18) and in 325 cases a concentrate stored for ≥5 days (incidence 1.78/100 transfusion-days; CI: 1.59 to 1.99) (table 1). The adjusted relative risk of all-cause bacteremia was 0.80 (CI: 0.58 to 1.12) after transfusion of a platelet concentrate stored for 3-4 days and 0.67 (CI: 0.49 to 0.92) after transfusion of a concentrate stored for ≥ 5 days, compared to transfusion of concentrates stored for ≤ 2 days, p value for trend: 0.007 (figure 2, crude analysis supplemental material).

Figure 2. Storage time and risk of all-cause bacteremia



Relative risk of all-cause bacteremia one day after transfusion of platelet concentrates stored 3-4 days or ≥5 days, compared to platelet concentrates stored ≤2 days, stratified on storage medium. Relative risks are adjusted for number of transfusions, AB0 blood group, day of the week, and hospital. Estimates for PAS stored platelet concentrates are also adjusted for generation of PAS.
Panel A) platelet concentrates stored in plasma or PAS
Panel B) platelet concentrates stored in PAS-B or PAS-C

Platelets in PAS

Among 1,527 patients receiving 11,822 PAS-stored platelet concentrates on 10,716 different days, 182 cases of bacteremia were detected the day after transfusion. In 31 cases the patient had received a platelet concentrate stored for ≤2 days (incidence 1.55/100 transfusion-days; CI: 1.06 to 2.20), in 90 cases a concentrate stored for 3-4 days (incidence 1.86/100 transfusion-days; CI: 1.50 to 2.29) and in 61 cases a concentrate stored for ≥5 days (incidence 1.57/100 transfusion-days; CI: 1.20 to 2.02) (table 1).The adjusted relative risk for developing a bacteremia was 1.14 (CI: 0.70 to 1.84) after transfusion of a platelet concentrate stored for 3-4 days and 1.19 (CI: 0.70 to 2.01) after transfusion of a concentrate stored for ≥5 days, p value for trend 0.545 (figure 2, crude analysis supplemental material table S3).

Additional analyses

For the first additional analysis only intensively treated patients were selected. In this subgroup of 333 cases receiving plasma-stored and 121 cases receiving PAS-stored platelet concentrates, results were similar to the entire cohort (supplemental material).

Second, subgroup analyses were performed for different generations of additive solutions. Storage time of platelet concentrates stored in PAS-B or PAS C was not associated with all-cause bacteremia (figure 2).

Third, the generation of additive solution was not associated with all-cause bacteremia (RR PAS-C versus PAS-B: 1.10, CI: 0.75 to 1.62)(table 2). Fourth, as a negative control, we selected cases the day before transfusion. In both storage media, storage time was not associated with the risk of all-cause bacteremia the day before transfusion (supplemental material).

Finally, we re-performed our analysis with an increased length of follow up. Storage time of platelet concentrates was not associated with all-cause bacteremia two and three days after transfusion (supplemental material).

Table 2. Generation of additive solution and risk of bacteremia

	Overall	≤2 days	3-4 days	5 days
Crude	1.11 (0.77-1.60)	0.90 (0.34-2.35)	1.21 (0.76-1.93)	1.00 (0.48-2.10)
Adjusted	1.10 (0.75-1.62)	1.02 (0.38-2.73)	1.20 (0.73-1.97)	0.93 (0.42-2.07)

Relative risk of all-cause bacteremia one day after transfusion of a platelet concentrate stored in PAS-C compared to PAS-B, stratified on storage time. The risk ratios are adjusted for number transfusions, AB0 blood group, day of the week, hospital, and storagetime.

Discussion

Transfusion of platelet concentrates stored ≥5 days in plasma, with 100% bacterial screening, was associated with a decreased risk of all-cause bacteremia the day after transfusion in patients with hematological malignancies. Storage time of platelet concentrates stored in PAS was not associated with all-cause bacteremia. For both storage media, storage time was not associated with all-cause bacteremia two or three days after transfusion. It is not known what role immunomodulation plays in producing the data we report.

Transfusion associated sepsis is often under-recognized and under-reported.[25] To capture all bacteremias, potentially related to a transfusion, we included all bacteremias the day after transfusion. We did not differentiate between various

potential causes of bacteremia and platelet concentrates were not re-cultured at time of transfusion. The incidences of bacteremia are higher than the incidence of infections exclusively caused by transfusion of a contaminated platelet concentrate. Active surveillance revealed an incidence of transfusion-transmitted infections ranging from 389 till 485 per million transfusions.[13,25] In our study the incidence of bacteremia was approximately 35 times higher, which would indicate that 14-17 of the 613 bacteremias after transfusion of a plasma stored platelet concentrate and 5 of the 182 bacteremias after transfusion of a PAS stored platelets are directly caused by contamination of the transfused products. This misclassification is not related to storage time and could therefore have biased the results towards the null (i.e. no association). The older storage time category contained relatively more transfusion days of patients with acute leukemia. Since these patients have the highest risk of infections, this could bias the results towards an increased risk of older platelets. However, we still found a lower risk of all-cause bacteremia after transfusion of older platelet concentrates stored in plasma. It is therefore exceedingly unlikely that the true effect is in the opposite direction. The lack of an association in the negative control supports our findings.

The assumed increased risk of bacteremia is one of the main arguments for limiting the shelf life of platelet concentrates.[26,27] The results of our study pertain all-cause bacteremia, which emphasizes all bacteremias and not exclusively transfusion-transmitted bacteremia, but based on these results, this argument seems at least unjustified regarding all-cause bacteremia for platelet concentrates stored in plasma when 100% bacterial screening is employed.

A limitation of this study, pertaining only to the results regarding PAS-stored platelet concentrates, is the limited number of cases, as only a subset of the hospitals used PAS stored platelet concentrates. For the majority of the study period, PAS-B stored platelet concentrates, which had a maximal storage time of only five days, were used. A limited range in possible storage time will automatically limit the differences. In several studies an association between platelet transfusions and risk of all-cause infection has been reported.[28-30] However, confounding by indication could be a potential explanation for these findings, since patients receiving platelet transfusions are at an inherently different risk of infection than those not receiving platelet transfusions. We here investigated differences in storage time, since platelet products are released on a first-in-first-out basis, without consideration of the patients' prognoses. During storage the risk of transfusion-transmitted infections increases [5,12,31] The effect of storage time on all-cause infections is less

studied and prior studies reported conflicted results.[32] One study reported an increased incidence of bacterial sepsis with each day increase of storage time in critically ill trauma patients.[33] Another study found no association between storage time of a single platelet concentrate and postoperative infections after cardiac surgery.[34] In contrast to these studies, we found a lower risk of all-cause bacteremia after transfusion of old platelet concentrates stored in plasma. This difference could possibly be explained by differences in platelet concentrate characteristics. In our study, platelet concentrates were buffy-coat derived and maximally stored for seven days, whereas in both other studies platelet concentrates were collected via apheresis and maximum storage time was limited to five days.

A higher incidence of contamination in fresh products could explain the lower risk of all-cause bacteremia after transfusion of longer stored platelet concentrates. With each day of storage the BacT/Alert will detect more contaminated products. However, the total incidence of positive screening results is only around 0.37% and this could not explain the total effect.[20] Moreover, platelet concentrates are cultured until the end of shelf-life. Approximately 80-100 units per year are transfused before the initial BacT/Alert turns out positive. Look-back procedures have shown that these only rarely lead to clinically significant infections.[35]

Another explanation for our results could be an immunomodulatory effect of platelet transfusions. Transfusion Related Immunomodulation (TRIM) has been studied in relation to red cell transfusions.[36] To which extend transfusion of platelets also modulate the immune response is less clear.[37] It has been shown *in vitro*, that levels of platelet-derived-growth factor and sCD40L (platelet activation factor) increase during storage.[38] In contrast, in mice, it has been suggested that fresh platelets have an immunosuppressive effect due to loss of the expression of MHC class I molecules during storage.[39] This would be in line with the increased incidence of all-cause bacteremia after transfusion of fresh platelet concentrates. We hypothesized that immune-modulatory effects of storage time of platelet transfusions probably last longer than one day. We therefore increased the time between transfusion and detection of bacteremia. However, we did not find an association between storage time and all-cause bacteremia after two or three days.

The lower risk of bacteremia after transfusion of older platelet concentrates stored in plasma was not observed for platelet concentrates stored in PAS. This could suggest that storage medium modifies the effect of storage time. It is known that not all bacteria are able to proliferate in platelet concentrates and some bacteria even die during storage, a process referred to as auto-sterilization.[40,41] This may be

more pronounced in platelet concentrates stored in plasma since plasma contains a mix of bactericidal proteins and enzymes.

Our study did not allow the comparison of risk of bacteremia with respect to storage medium itself. The lower incidences of bacteremia after transfusion of PAS stored platelet concentrates may suggest a beneficial effect of PAS. However, although storage medium was solely determined by geographic location of the hospital, the included type of hospitals and thereby also the type of patients differed substantially between the different storage media. These substantial differences hamper a direct comparison of storage media and we did not attempt to adjust for this confounding.

In conclusion, in patients with hematological malignancies, storage time of plasma-stored platelet concentrates was associated with a decreased occurrence of all-cause bacteremia the day after transfusion, whereas storage time was not associated with the incidence of all-cause bacteremia the day after transfusion of PAS-stored platelets.

Supplemental material

Available at http://onlinelibrary.wiley.com/doi/10.1111/trf.14194/abstract

Table S1. Participating hospitals
Table S2. Selected DBC codes for hematological malignancies
Table S3. Storage time and risk of all-cause bacteremia
Table S4. Storage time and risk of all-cause bacteremia one day after transfusion in most intensively treated patients
Figure S1. Flow chart of data handling
Figure S2. Storage time of platelet concentrate stored in PAS –B and PAS-C for cases and controls
Figure S3. Storage time and risk of all-cause bacteremia the day before transfusion
Figure S4. Storage time and risk of all-cause bacteremia 2 or 3 days after transfusion

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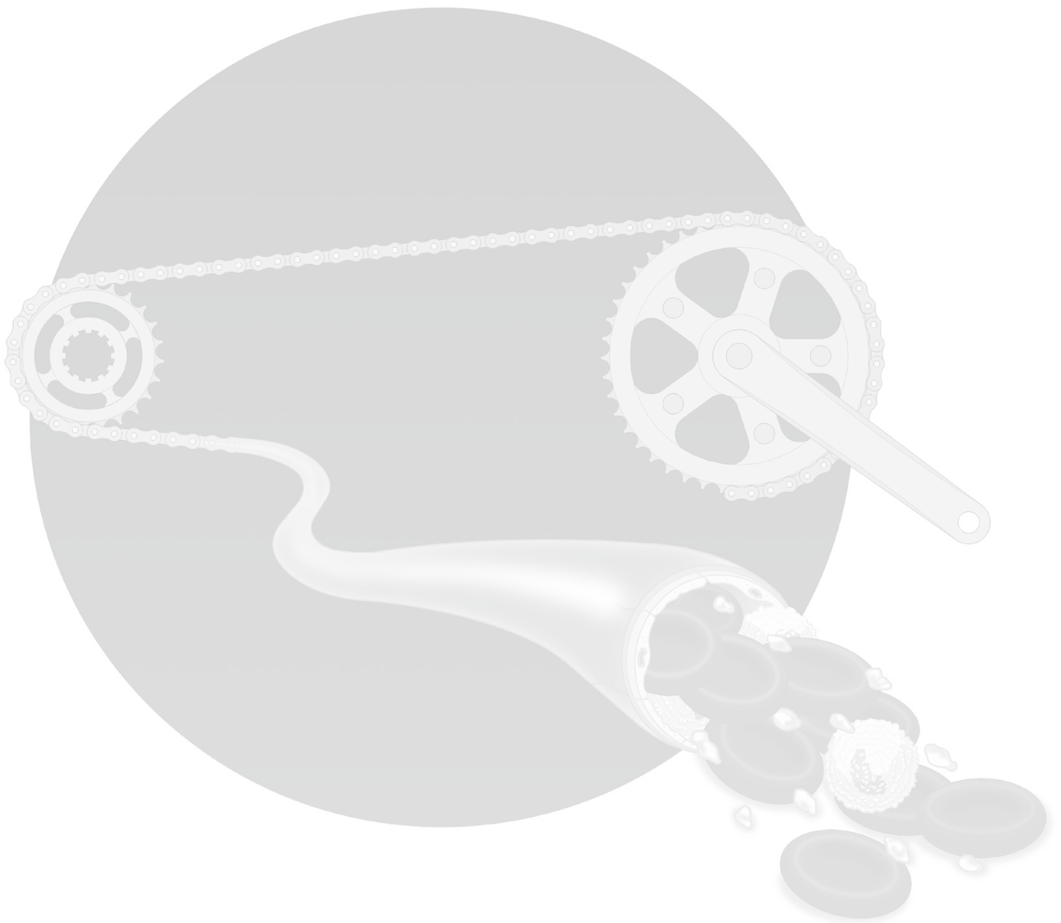

Chapter 8

Storage time of platelet concentrates and risk of a positive blood culture; A nationwide cohort study

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Abstract

Background

Concern of transfusion-transmitted bacterial infections has been the major hurdle to extend shelf life of platelet concentrates. We aimed to investigate the association between storage time and risk of positive blood cultures at different times after transfusion.

Methods

We performed a nationwide cohort study among recipients of platelet transfusions in Denmark between 2010 and 2012, as recorded in the Scandinavian Donations and Transfusions (SCANDAT2) database. Linking with a nationwide database on blood cultures (MiBa), we compared the incidence of a positive blood culture among recipients of platelets stored six to seven days (old) to those receiving fresh platelets (one to five days), using Poisson regression models. We considered cumulative exposures in windows of one, three, five, and seven days.

Results

A total of 9,776 patients received 66,101 platelet transfusions. The incidence rate ratio of a positive blood culture the day after transfusion of at least one old platelet concentrate was 0.77 (CI 0.54-1.09) compared to transfusion of fresh platelet concentrates. The incidence rate of a positive blood culture was lower the day after receiving one old compared to one fresh platelet concentrate (IRR 0.57; CI: 0.37-0.87). Three, five, or seven days after transfusion, storage time was not associated with the risk of a positive blood culture.

Conclusion

Storage of buffy coat derived platelet concentrates in PAS-C up to seven days seems safe regarding the risk of a positive blood culture. If anything, transfusion of a single old platelet concentrate may decrease this risk the following day.

Introduction

Platelet concentrates are transfused to prevent or treat bleeding complications in patients with low platelet count or severe platelet dysfunction. In contrast to other blood components, platelet concentrates are stored at room temperature which may facilitate bacterial growth.[1] Bacterial sepsis caused by transfusion of contaminated blood products currently constitutes the largest transfusion-associated infectious risk.[2] In many blood centers, platelet concentrates are screened for bacterial contamination in an attempt to reduce this risk.[3,4] However, such screening is costly and is limited by false negative test results.[2,5]

Because most reported septic transfusion reactions were associated with platelet concentrates stored for 4 days or more, older platelet concentrates are believed to increase the risk of transfusion-associated bacterial infections.[6-9] A reduction of the maximum permitted storage time of platelet concentrates could conceivably reduce this risk. Therefore, storage time has been limited to 3.5 days in Japan and 4 days in Germany.[4,10] Such a strategy might increase rates of product outdating and limit the number of components in stock to cope with emergency situations.[11] In several countries, including the Netherlands and Denmark, platelet concentrates can be stored for up to seven days in combination with bacterial screening.[4]

Besides a direct risk of transfusion-transmitted bacterial infections, platelets could also modulate the immune response and thereby influence the risk of infections. During storage several cytokines and chemokines, which could have immunomodulatory effects, are released.[12]

We have previously shown that the overall risk of bacteremia of any cause was decreased in hematological patients one day after transfusion of platelet concentrates stored five to seven days as compared to patients who received units stored one or two days. The association was limited to transfusion of platelet concentrates stored in plasma, whereas storage time was not associated with the risk of all-cause bacteremia when the platelets were stored in platelet additive solution (PAS). However, the power of the latter analysis was limited by the sample size for platelets stored in PAS-C and the maximal storage time of five days for platelets in PAS-B.[13]

In Denmark, all platelet concentrates are stored in PAS-C with a maximal storage time of seven days. The current study aimed at investigating the effect of storage for six or seven days on risk of a positive blood culture at different times after

transfusion in all recipients of a platelet transfusion by using administrative health care data.

Methods

Setting

We performed a nationwide cohort study among all patients receiving platelet transfusions in Denmark between 2010 and 2012. For the purpose of the present study, we restricted the study population to patients who were 18 years or older at transfusion. To ensure a homogeneous patient population, hospitals administering less than 1000 platelet transfusions during the study period were excluded. The study was approved by the Danish Data Protection Agency (2015-57-0012).

Data source

We obtained information on transfusion recipients and blood components from the Scandinavian Donations and Transfusions database (SCANDAT2), which has been described in detail elsewhere.14 In brief, data on donations and transfusions were collected from blood banks covering all of Sweden and Denmark. Data were linked to national registers of migration, death, and hospital care, using the unique personal identification number assigned to all residents of Sweden and Denmark. Recipient data included information on sex, blood group, dates of birth, death and migration, discharge diagnoses, and procedure codes. Data on blood components included date of donation and transfusion, type of blood component, and blood group of donor(s). For the current study we selected the transfusions to Danish residents. Information on blood culture results was obtained from MiBa, the Danish microbiology database.15 MiBa contains copies of reports from all Danish departments of microbiology with a sampling date between 1 January 2010 and 31 December 2012. Blood cultures were taken on clinical indication or routinely as part of certain treatment protocols, not necessarily directly related to the transfusion. This information was linked to the transfusion data via the personal identification number.

Exposure

Platelet concentrates were produced from buffy coats of four ABO and Rhesus D matched donors and re-suspended in platelet additive solution (PAS-C, Intersol, Fenwal ™). Buffy coats are pooled 3 to 30 hours after donation, but preferably after overnight hold of whole blood. All platelet concentrates were screened for bacterial contamination using the BacT/Alert system, which consists of an aerobic bottle and is inoculated with 5-10 ml. Sampling is performed right after pooling of the buffy

coats.[4] Products are released according to a 'negative-to-date' procedure. Maximum storage time of platelet concentrates was seven days. Storage time was counted in days from the day of donation (day 0) up to and including the day of transfusion. Platelet concentrates stored for six or seven days were considered 'old' and platelet concentrates stored for one to five days were considered 'fresh'. Approximately 3% of all platelet concentrates were collected via apheresis. These products were taken into account when adjusting for total number of transfusions, but we did not study storage time of apheresis products, as most of these were given for specific indications.

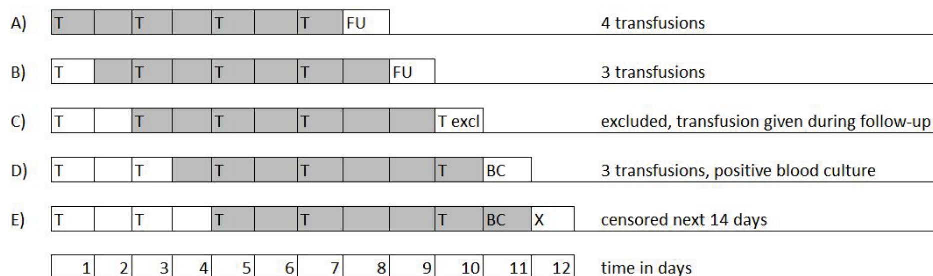
Outcome

The primary outcome of interest was a positive blood culture, regardless of the cause. Patients could develop a positive blood culture multiple times during the study period. Two consecutive positive cultures were considered to be unrelated if separated by at least 14 days.

Statistical analysis

The main analysis tested whether the occurrence of a positive culture on a given day was associated with transfusion of at least one old platelet unit during the preceding one to seven days. We employed a sliding window approach, with an exposure ascertainment period of one, three, five, or seven days with a subsequent 1-day follow-up period during which we ascertained the occurrence of a positive blood culture (figure 1). Patients were considered at risk if they received at least one platelet concentrate during the window period and did not have a positive blood culture within the previous 14 days. For each day of follow-up we then advanced both the exposure and outcome ascertainment periods one day at a time. Because both transfusions and blood cultures were only recorded per calendar day, it was not possible to know whether blood cultures were drawn before or after a transfusion. We therefore excluded follow-up on days on which a transfusion was given.

Figure 1. Examples of cumulative exposure during a seven-day window period



A) All transfusions during the window period are counted. Day 8 is the day of follow-up.
B) The next window period starts one day later.
C) If a transfusion is given at the follow-up day, this window period is excluded.
D) Positive blood culture at day of follow-up
E) After a positive blood culture patients are censored for 14 days.

The incidence rate of a positive blood culture after transfusion of at least one old platelet concentrate was compared with the incidence rate after transfusion of only fresh platelet concentrates, using Poisson regression. The analyses were adjusted for day of the week, rhesus D antigen positivity of the product, and hospital, using stratification of person time. Day of the week and rhesus D antigen positivity were treated as time-dependent variables, based on the last transfusion given during that window period. We did not adjust for any patient characteristic, as storage time is not known by the treating physician and therefore confounding by indication is unlikely to arise. Robust variance estimates were used, as patients could contribute more than one window period of which each may be terminated by a positive blood culture.[16]

The number of platelet transfusions a patient received during a window period could confound the association between storage time and risk of a positive blood culture, as number of transfusions is a strong indicator of sickness of the patient and risk of receiving at least one old platelet concentrate. Therefore, we stratified on number of platelet transfusions: one, two, three, and four or more transfusions during the window period. For the one-day window period analyses were stratified on one, two, and three or more transfusions.

Additional analyses

We performed three additional analyses. First, we assessed the effect of receiving at least one old platelet concentrate in a subgroup of patients with a hematological malignancy or aplastic anemia. This subgroup was established using the sequential algorithm used in previous studies, based on diagnosis and procedure codes.[17-19] Here we did not differentiate between main and co-diagnoses in the hospital register data. The covariates and stratification were the same as in the main analysis. Second, to test for a dose-response relationship, we modeled the number of old products as main exposure, stratified on number of platelet transfusions and adjusted for the same confounders as in the main analysis.

Third, we included also transfusions given during the follow-up days in the analysis to investigate whether we introduced selection bias by excluding these days. Specifically, it has been suggested that the time to the next transfusion is shorter after transfusion of an old platelet concentrate than after transfusion of a fresh concentrate.[20-22] Therefore, we could have excluded more follow-up time, and thereby probably more events, after transfusion of old platelet concentrates, which could have introduced selection bias.

All statistical analyses were conducted using SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina), the GENMOD procedure. For stratification and aggregation of follow-up time the stratify macro was used.[23]

Results

Patient characteristics

Between 2010 and 2012, a total of 12,529 patients received at least one platelet transfusion in Denmark. Of these, 826 patients were excluded based on age at time of transfusion and 1,927 patients were excluded as they only received a transfusion in a hospital that accounted for fewer than 1000 platelet transfusions in the study period. The final cohort consisted of 9,776 patients, more men than women (62.3% versus 37.7%), with an average age of 64.1 years (table 1). During the study period, these patients received 66,101 platelet transfusions, of which 22,240 units (33.6%) were stored for six or seven days. This relatively large proportion of old platelet concentrates is a consequence of the first-in-first-out policy. Forty-nine percent of all platelet concentrates were transfused to patients with a hematological malignancy, 15.6% to patients with trauma or burns, and 8.6% to patients who underwent cardiothoracic surgery. The distribution of diagnoses was similar among the storage time categories. Information about blood group of the product was missing for 11,156 products, but this was equally distributed among the storage

time categories. The proportion of rhesus D negative products increased with increasing storage time (Table 2).

Table 1. Characteristics of study population

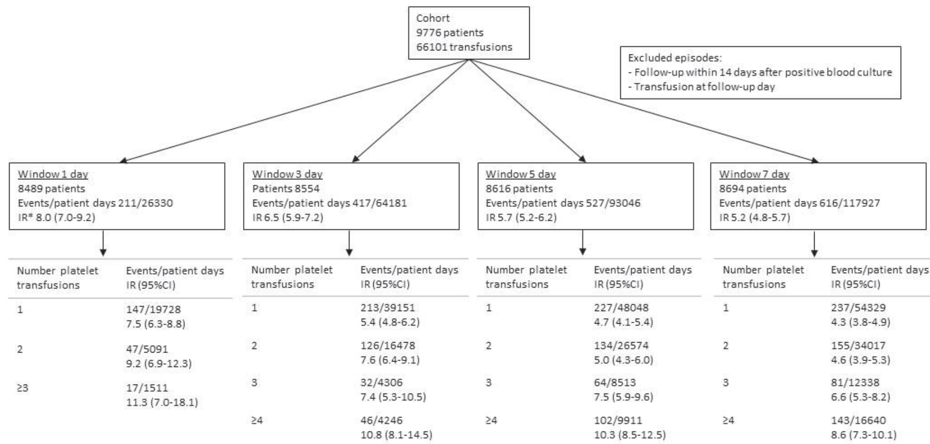
	Number of patients (%)
Patients, n	9,776
Male, n (%)	6,088 (62.3)
Age, n (%)	
18-49 years	1,533 (15.7)
50-74 years	6,011 (61.5)
≥75 year	2,232 (22.8)
Mean (SD) age in years	64.1 (14.6)
Median (IQR) number of transfused platelet concentrates	2 (1-6)
Median (IQR) number of transfused red blood cell concentrates	12 (5-24)
Median (IQR) number of transfused plasma products	2 (0-8)

Table 2. Characteristics of transfused platelet concentrates

	Storage time 1-5 days	**Storage time 6 -7 days**	**Total**
Number of platelet concentrates (%)*	34,722 (52.5)	22,240 (33.6)	66,101 (100)
Male, n(%)†	21,628 (62.3)	14,151 (63.6)	41692 (63.1)
Mean (SD) age in years†	60.3 (14.9)	60.0 (15.1)	60.2 (14.9)
Median (IQR) number of prior transfusions all products	32 (9-79)	33 (9-83)	34 (10-84)
Main indication			
Hematology	17,029 (49.0)	10,681 (48.0)	32,547 (49.2)
Cardiothoracic surgery	3,017 (8.7)	1,914 (8.6)	5,657 (8.6)
Trauma and burns	5,407 (15.6)	3,429 (15.4)	10,319 (15.6)
Bleeding	1,177 (3.4)	675 (3.0)	2,106 (3.2)
Unknown	8,092 (23.3)	5,541 (25.0)	15,472 (23.4)
Donor ABO blood group, n (%)			
A	13,958 (40.2)	8,019 (36.1)	22,377 (33.9)
B	1,551 (4.5)	510 (2.3)	2,084 (3.2)
AB	9 (0.03)	1 (0.0)	15 (0.0)
O	16,736 (48.2)	12,216 (54.9)	30,469 (46.1)
Rhesus antigen positivity, n (%)	26,949 (77.6)	14,094 (63.4)	42,065 (63.6)
Missing blood group, n (%)	2,468 (7.1)	1,494 (6.7)	11,156 (16.9)

** Percentages do not add up until 100%. 'Total' also includes apheresis products (2.7%) and products with unknown storage time (11.1%). †per number of platelet concentrates*

Figure 2. Crude estimates of incidence rates of a positive blood culture after transfusion of 1, 2, 3 and ≥4 platelet concentrates during different window periods.



The number of analyzed patients and the total follow-up time differed among the window periods, since patients were only considered at risk if they received at least one platelet concentrate during the window period and no transfusion at the day of follow-up. This means that in the window period of one day, a transfusion contributed only to one window period and one day of follow-up was counted. In a sliding window period of seven days with steps of one day, a day of transfusion contributed to seven subsequent window periods, and seven days of follow-up could be counted.
**IR (CI), incidence rate, expressed per 1000 patient-days of follow-up.*

<u>Incidence of positive blood cultures</u>

The day after transfusion 211 cases of positive blood cultures occurred, which corresponds to an incidence rate of 8.0 per 1000 observation days (95% confidence interval (CI) 7.0 to 9.2). Among patients receiving a transfusion within the last three days, the incidence rate was 6.5 per 1000 days (95% CI 5.9 to 7.2). Considering a five day window period the incidence rate was 5.7 per 1000 days (95% CI 5.2 to 6.2) and for the seven day window period this was 5.2 per 1000 days (95% CI 4.8 to 5.7). The incidence rate increased with an increasing number of platelet transfusions in all window periods (figure 2).

Old versus fresh platelet concentrates

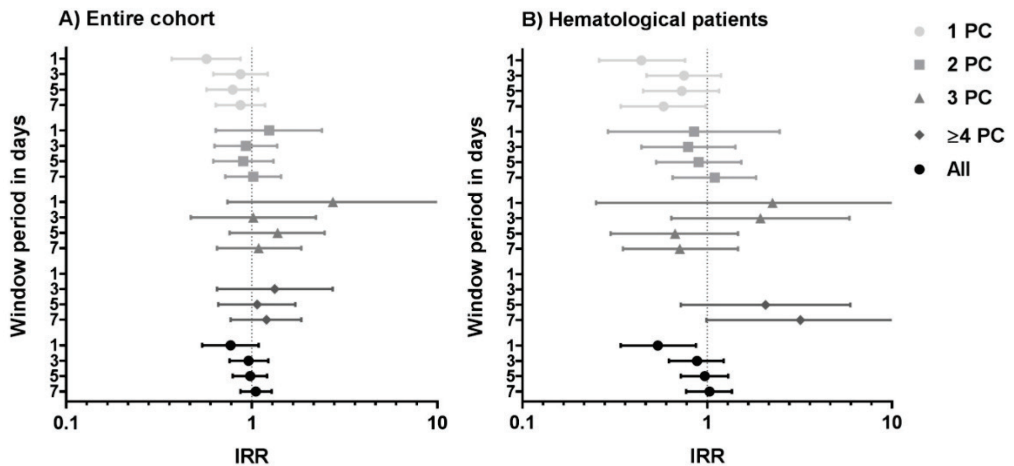
Figure 3 presents the incidence rate ratios (IRR) of a positive blood culture after transfusion of at least one old platelet concentrate, compared to only fresh platelet concentrates, for all window periods, stratified on total number of platelet transfusions. The incidence rate ratio of a positive blood culture the day after transfusion of at least one old platelet concentrate compared to only fresh platelet concentrates was 0.77 (95% CI 0.54 to 1.09). Considering a window period of three days, the incidence rate ratio of a positive blood culture was 0.96 (95% CI 0.76 to 1.23). This was 0.98 (95% CI 0.79 to 1.21) for a five day window period and 1.05 (95% CI 0.87 to 1.28) for a seven day window period (supplemental material).

For patients receiving a single platelet concentrate, the incidence rate of a positive blood culture the day after transfusion was lower if this was an old platelet concentrate (IRR 0.57; 95% CI 0.37-0.87). This association was not statistically significant if the old platelet concentrate was transfused in the preceding three, five or seven days (figure 3).

Additional analyses

For patients with a hematological malignancy or aplastic anemia, the incidence rate ratio of bacteremia the day after transfusion of at least one old platelet concentrate was 0.54 (CI 0.31 to 0.87) compared to transfusion of only fresh platelet concentrates. After receiving a single old, compared to a single fresh, platelet concentrate the incidence rate ratio was 0.44 (95% CI 0.26 to 0.76). If a patient received one old platelet concentrate in the preceding three, five, or seven days, the estimates were similar, although not statistically significant (figure 3, panel B and supplemental material).

Figure 3. Incidence rate ratio of a positive blood culture after receiving at least one old platelet concentrate compared to transfusion of only fresh platelet concentrates.



IRRs with 95% confidence interval are presented overall and stratified by number of total platelet transfusions during a window period of one, three, five, and seven days. If a patient received several platelet concentrates during the window period, no differentiation was made whether only one or more products were old. Incidence rate ratios are adjusted for hospital, rhesus D blood group, and day of the week. Overall estimate is also adjusted for number of platelet transfusions (1, 2, 3, ≥4).
Panel A) Entire cohort.
Panel B) Patients with hematological malignancy or aplastic anemia
The corresponding numbers are given in the supplemental material.

There was no evidence of a dose-response relationship (table 3). For patients who received two transfusions, the incidence rate ratio for a positive blood culture the day after receiving exclusively old platelet concentrates compared to exclusively fresh platelet concentrates was 1.11 (95% CI 0.53 to 2.31). Similar estimates were observed for patients receiving three, or four or more platelet concentrates and if these transfusions were given during a longer window period (table 3). Including the follow-up days on which a patient had received a platelet transfusion did not change the results (supplemental material).

Table 3. Incidence rate ratio for positive blood culture per number of old platelet concentrates, stratified on total number of platelet transfusions during a window period of one, three, five, or seven days.

Total number of PLT transfusions	Number of old PLT concentrates	Window period			
		1 day	3 days	5 days	7 days
1	0	Reference	Reference	Reference	Reference
1	1	0.57 (0.37-0.87)	0.87 (0.62-1.22)	0.79 (0.57-1.08)	0.87 (0.64-1.18)
2	0	Reference	Reference	Reference	Reference
2	1	1.54 (0.64-3.71)	0.87 (0.53-1.44)	0.92 (0.58-1.43)	1.08 (0.72-1.63)
2	2	1.11 (0.53-2.31)	0.98 (0.62-1.54)	0.88 (0.55-1.42)	0.95 (0.62-1.47)
3†	0	Reference	Reference	Reference	Reference
3†	1	2.39 (0.40-14.31)	1.05 (0.39-2.81)	1.30 (0.65-2.61)	1.09 (0.59-2.02)
3†	2	5.10 (1.13-23.00)	1.87 (0.75-4.63)	1.88 (0.97-3.65)	1.24 (0.65-2.36)
3†	3	1.94 (0.43-8.71)	0.21 (0.03-1.63)	0.79 (0.29-2.18)	0.87 (0.36-2.09)
≥4	0	NA‡	Reference	Reference	Reference
≥4	1	NA‡	1.10 (0.35-3.41)	1.18 (0.62-2.23)	1.07 (0.59-1.91)
≥4	2	NA‡	1.40 (0.52-3.78)	0.84 (0.42-1.68)	1.08 (0.61-1.91)
≥4	3	NA‡	0.76 (0.17-3.42)	1.03 (0.49-2.14)	1.26 (0.68-2.33)
≥4	≥4	NA‡	1.64 (0.70-3.80)	1.20 (0.66-2.17)	1.37 (0.81-2.33)

* IRRs with 95% CIs are adjusted for hospital, D blood group, and day of the week. Transfusion of only fresh PLT concentrates were used as reference. Incidences are depicted in Table S2, available as supporting information in the online version of this paper.

† For the 1-day window period. The total number of PLT transfusions is three or more, as almost no patients received four or more transfusions within 1 day.

‡ NA = not applicable. Insufficient number of patients received four or more transfusions in the 1-day window period.

Discussion

In this nationwide cohort study, transfusion of platelet concentrates stored for six or seven days was not associated with an increased risk of a positive blood culture compared to transfusion with platelet concentrates stored for five days or less. If anything, risk of a positive blood culture was lower the day after transfusion of one old platelet concentrate compared to one fresh platelet concentrate.

Storage time was only associated with a lower incidence of a positive blood culture after transfusion of a single platelet concentrate. The lack of an association when patients had received multiple transfusions might conceivably be attributable to effect modification by indication or underlying morbidity. Specifically, diagnoses and indications might differ between patients who need only one transfusion and those who need more transfusions. As corollary, the patients who needed the most transfusions might also have a higher baseline risk of infection. The increased incidence of a positive blood culture with an increasing number of transfusions, as observed in the present study, would be compatible with this notion. Hence, under such circumstances any variation in infection risk by storage time of a transfused product might be clinically irrelevant and immeasurable. In addition, the groups receiving multiple transfusions were smaller which limits the power to detect such small effects.

The protective effect of an old platelet concentrate was only seen after the shortest window period. Most hematological patients receive prophylactic antibiotics, which could result in negative blood cultures after three or more days, but maybe not immediately the day after transfusion. The higher risk of a positive blood culture soon after transfusion of fresh platelets could be due to contamination which was not yet detected by the bacterial screening system. Not all bacteria are able to proliferate within a platelet concentrate, so during storage a blood component could auto-sterilize.[2,24,25] However, studies have shown that platelet concentrates transfused before the screening turned out positive only marginally increase the risk of clinically significant infections.[26,27] Another postulated theory is that platelets play a role in the immune system and transfusions could modulate this response.[12,28,29] During storage, efficacy of platelets reduces: referred to as 'the storage lesion'.[30,31] This may imply that older platelets are not consumed immediately in hemostatic activities and still exert a relatively higher activity of non-hemostatic functions that may protect the patient better against infections.

The reduction in efficacy during storage could also have introduced selection bias, as we excluded follow-up time in which a patient received a transfusion and the interval between transfusions may be shortened after transfusion of old platelet concentrates.[32] However, the sensitivity analysis including follow-up time in which a patient received a transfusion yielded similar results.

A major strength of our study is that we were able to study storage of platelet concentrates for up to seven days. In many countries storage is limited to five days, but blood banks worldwide are seeking to extend their maximum storage time.[33] The draft guidance of the FDA stated that transfusion of platelet concentrates stored for six or seven days is allowed, provided that these concentrates are cultured again on day four or five of storage, or rapid testing is performed within 24 hours prior to transfusion. However, no culture system has been certified up to now.[34]

Studies regarding transfusion-associated sepsis are often based on data gathered by passive surveillance. It has been suggested that such a strategy underestimates the true incidence as much as 40-fold.[35,36] We included all positive blood cultures as a surrogate outcome to overcome this underestimation. Such a strategy implicates that we also included blood cultures that were positive due to contamination of the culture and not the result of a bacteremia accompanied by clinically relevant symptoms. Bacteria identified in contaminated blood cultures are often skin derived and the same as those identified in contaminated blood products and transfusion-transmitted infections.[27] It is therefore impossible to distinguish between these. As a consequence, the incidence in our study overestimates the true incidences of all-cause bacteremia and transfusion transmitted bacterial infections. As contamination of blood cultures is unrelated to storage time of platelet concentrates, this misclassification may have biased the results toward the null, meaning no association. Moreover, since we used positive blood cultures as a surrogate outcome for bacteremia, we were unable to completely rule out the potentially fatal residual risk of septic transfusion reactions after screening. This especially accounts for older products. Sampling for the BacT/Alert is performed 24 hours after donation, so initial low inocula could be missed, resulting in false negative screening. Proliferation during storage may result in a high bacterial load at time of transfusion and an increased risk of severe septic transfusion reactions.[37,38] The same kind of bias applies to transfusion of other blood products. It has been suggested that red cell transfusions also have immunomodulatory effects. However, transfusion of other blood products is not associated with storage time of platelet concentrates.[32,39] Therefore, these additional transfusions could not

confound our results.
These biases could potentially explain the lack of an association in patients who received multiple platelet concentrates. However, they are unlikely explanations of our observation of a protective effect of transfusion of a single old platelet concentrate on the incidence of positive blood cultures.

The present findings are consistent with the results of our previous study. In a population of Dutch hematological patients, the risk of bacteremia was lower the day after transfusion of platelet concentrates stored in plasma for five to seven days compared to those stored one or two days.[13] In the Dutch study, we only included days on which a patient received platelet concentrates exclusively of a single storage time category. On most analyzed days, patients received only one transfusion. Therefore, these results are comparable with the conclusion of the current study regarding transfusion of a single old platelet concentrate. Since we now have found a similar effect in two independent cohorts, using different methods, it is unlikely that this association has arisen from chance alone.

To conclude, regarding the risk of a positive blood culture, it seems to be safe to store platelet concentrates up to seven days in combination with 100% screening. Transfusion of a single old platelet concentrate may decrease the risk of a positive blood culture the day after transfusion, especially in patients with a hematological malignancy.

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Supplemental material

Available at http://onlinelibrary.wiley.com/doi/10.1111/trf.14401/abstract

Table S1. Incidence rate ratio of a positive blood culture with 95% confidence interval after receiving at least one old platelet concentrate compared to transfusion of only fresh platelet concentrates during a window period of 1, 3, 5, or 7 days

Table S2. Number of events per number of patient days after transfusion of 1, 2, 3 or ≥4 or more old platelet concentrates, stratified per total number of transfusions during a window period of one, three, five, or seven days.

Table S3. Incidence rate ratio with 95% confidence interval of a positive blood culture after receiving at least one old platelet concentrate compared to transfusion of fresh platelet concentrates, including the follow-up days on which patient had received a transfusion.

Figure S1. Incidence rate ratio with 95% confidence interval of a positive blood culture after receiving an old platelet concentrate compared to transfusion of fresh platelet concentrates, including the follow-up days on which patient had received a transfusion.

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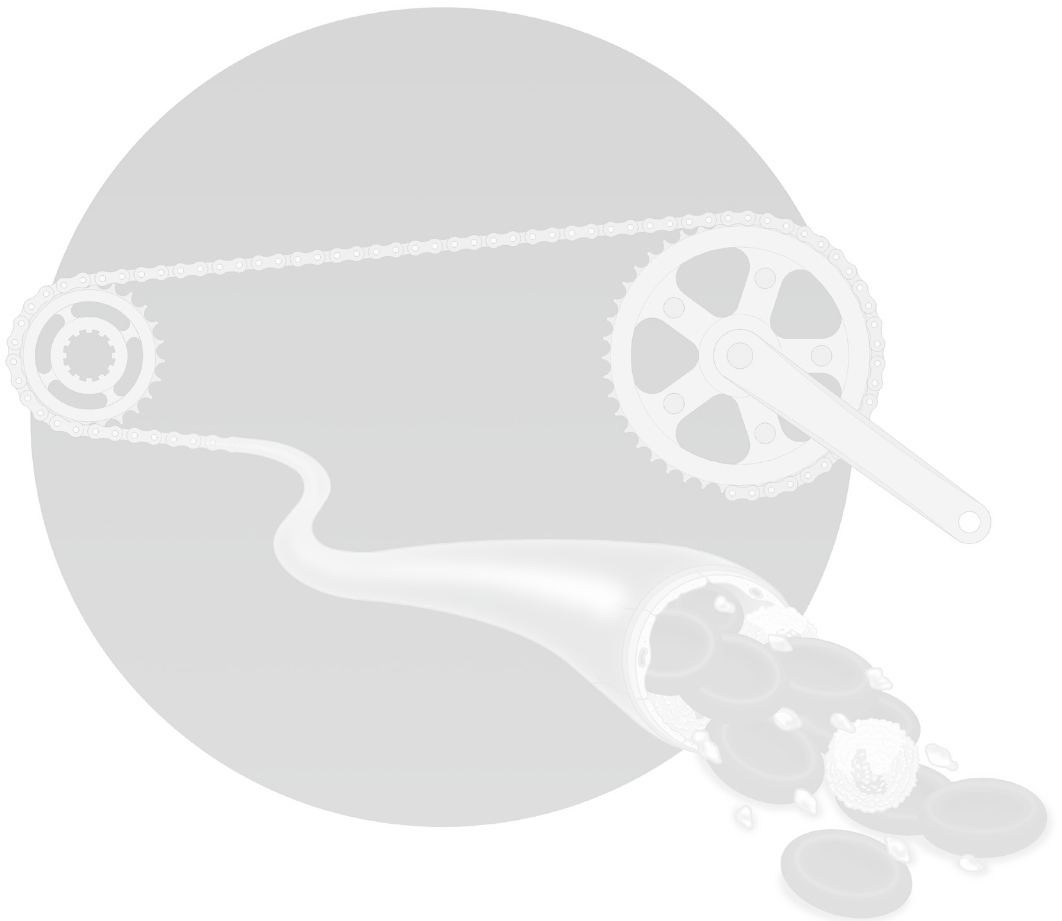

Chapter 9

Storage medium of platelet transfusions and the risk of transfusion transmitted bacterial infections

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Abstract

Transfusion transmitted bacterial infections (TTBI) are among the most concerning risks of transfusion of platelet concentrates. Storage medium influences bacterial growth dynamics and thereby the sensitivity of screening tests for bacterial contamination. The aim of this study was to quantify the association of storage media with the incidence of TTBI after transfusion of platelet concentrates. In the Netherlands, the choice of storage medium is determined solely by geographic location of the hospital. We compared types of storage medium of all reported cases of TTBI following transfusion of a platelet concentrate with types of storage medium of all produced platelet concentrates in the Netherlands from 2003 to 2014. Fourteen cases of TTBI were reported, of which 57.1% received a platelet concentrate stored in platelet additive solution (PAS) and 42.9% a platelet concentrate stored in plasma. Of all produced platelet concentrates 22.3% were stored in PAS and 77.7% in plasma. The relative risk of TTBI after transfusion of a PAS stored platelet concentrate was 4.63 (95% confidence interval (CI) 1.4 to 16.2) compared to transfusion of a plasma stored platelet concentrate. The incidence of TTBI was 22.2 per million (CI 12.1 to 37.2 per million) transfused buffy coat platelet concentrates.

Introduction

Transfusion transmitted bacterial infections (TTBI) are one of the leading causes of mortality associated with blood transfusion.[1] Risk of TTBI is particularly associated with transfusion of platelet concentrates, as these are stored at room temperature, allowing for proliferation of bacteria.

In many countries, platelet concentrates are screened for bacterial contamination, using the BacT/Alert culture system, and released on a 'negative-to-date' basis.[2] Despite preventive efforts, still a significant number of TTBIs are reported every year. With complete bacterial screening, the incidence of TTBI was 7.14 per million platelet transfusions in Germany between 1997 and 2007, and 9.14 per million in the USA (2007-2011).[3,4] Approximately 300,000 platelet concentrates are transfused yearly in the United Kingdom and in 2015 the first case since 2009 was reported.[5] In the absence of bacterial screening, the incidence of TTBI was 26.5 per million In France (2009-2011).[6]

Sensitivity of the screening method is influenced by variability in the inoculum and kinetics of bacterial growth.[7] Bacteria have been shown to be present in higher concentrations, making them more likely to be detected by culture methods, in apheresis and buffy coat derived platelet concentrates stored in platelet additive solution (PAS), as compared to those stored in plasma.[8-10]

Interestingly, for some products yielding a positive BacT/Alert screen, a subsequent resampling of the stored platelet concentrate results in a negative culture.[11] Apparently not all bacteria are able to proliferate in a platelet concentrate. It has been suggested that complement and antibodies can eliminate bacteria and sterilize the blood product. This process of auto-sterilisation is probably more pronounced in platelet concentrates stored in plasma than in those stored in PAS.[12]

It is not known how these different effects of storage media influence the total risk of TTBI. The aim of this study was to quantify the association of storage medium with the incidence of TTBI after transfusion of a platelet concentrate.

Methods

We performed a nested case control study to assess the effect of storage of platelet concentrates in plasma or PAS on the risk of TTBI. We included all cases of TTBI in which a platelet transfusion was involved that had been reported to the national hemovigilance organization 'Transfusion and Transplantation Reactions in Patients'

(TRIP) between 2003 to 2014. TRIP is the Dutch competent authority to which all transfusion reactions must be reported. Product identification numbers of the involved products were used to extract information about storage media and production method from the blood bank system. We excluded cases of TTBI that occurred after transfusion of platelet concentrates collected by apheresis for the main analysis, because these are used for specific indications and mostly stored in plasma.

TTBI was defined as clinical features of bacteremia or sepsis during or after transfusion, with a relevant positive blood culture in the patient and assessed with a high level of imputability (definite or probable) to the transfused product. Imputability of all cases of post-transfusion sepsis was assessed by an expert panel. Since 2011 the expert panel has additionally judged whether the bacterial culture findings support a formal classification of the case as TTBI. Severity of transfusion reactions was scored on a scale from 0 to 4, with 0 indicating 'no morbidity' and 4 indicating 'mortality'.[13]

Platelet concentrates were prepared from buffy-coats of five donors, leukoreduced, and resuspended in plasma, or platelet additive solution (PAS), with 25 ml of plasma left per donor. PAS-B (T-sol, Baxter) was used through 2013, with PAS-C (Intersol, Fenwal, Inc) being used since. The diversion pouch was introduced universally in July 2004.[14] Throughout the entire study period, a standardized skin disinfection method was used and all platelet concentrates were screened for bacterial contamination with the BacT/Alert system (bioMérieux), according to a standardized protocol.

For the incidence of TTBI the number of all platelet concentrates produced in the Netherlands between 2003 and 2014 was used as the denominator. The storage medium of platelet concentrates involved in a TTBI was compared to storage medium of all produced platelet concentrates. Production data according to storage medium were available only for the period 2006-2014. The ratio of used storage media was stable over this period and could therefore be extrapolated back to 2003 (supplemental material). The type of storage medium of platelet concentrates is only determined by the geographical location of the hospital. Therefore location of the hospital where the case of TTBI arises behaves as an instrumental variable in this analysis and it is expected that all potential confounders are randomly distributed.[15] To assess this assumption we explored the distribution of storage medium among hospitals licensed for stem cell transplantations and we compared the incidences of transfusion reactions related to red blood cell transfusions between the regions. We performed two sensitivity analyses. First, we included apheresis products in our

analysis. Second, we excluded all cases before July 1st 2004, when use of the diversion pouch was introduced in all production centres.

Results and discussion

Between 2003 and 2014 fourteen cases of TTBI were reported to TRIP. Table 1 provides the characteristics of all these cases. One case was of minor severity (grade 1), ten cases were moderate to serious (grade 2), one was directly life-threatening (grade 3), and one was fatal (grade 4). Twelve patients had a hematological malignancy, one patients had a solid tumour (prostate carcinoma) and for one patient the indication for transfusion was stated to be thrombocytopenia without further reported diagnosis. Both cases in 2003 were related to Bacillus Cereus. The bacterial strains differed in genotype, so it seemed unlikely that both platelet concentrates were contaminated by a common source.[16]

During the study period 631,347 pooled buffy coat platelet concentrates were produced. The incidence of TTBI was 22.2 per million (95% confidence interval (CI) 12.1 to 37.2 per million) buffy coat platelet concentrates. This incidence is relatively high compared to other countries, which is probably a reflection of the accuracy of the Dutch hemovigilance system.[17]

Eight patients (57.1%) with TTBI received a PAS stored platelet concentrate (seven PAS-B, one PAS-C) and six patients (42.9%) received a platelet concentrate stored in plasma. Of all produced platelet concentrates, 22.3% were stored in PAS, and 77.7% in plasma. Transfusion of PAS stored platelet concentrates was associated with a relative risk of TTBI of 4.63 (95% CI 1.4 to 16.2) compared to plasma stored platelet concentrates. Including the platelet concentrates collected via apheresis showed similar results (RR 5.01; CI 1.66 to 15.83). Exclusion of the period before universal use of the diversion pouch yields a relative risk of 3.48 (CI 0.93 to 13.01).

Table 1. All cases of TTBI reported to TRIP between 2003 and 2014

Case	Year	Age in years	Diagnosis	Severity*	Bacteria	Storage medium
1	2003	18	Acute myeloid leukemia	2	Bacillus Cereus	PAS-B
2	2003	57	Chronic myeloid leukemia	N/A†	Bacillus Cereus	PAS-B
3	2004	28	N/A†	2	Bacillus Cereus	PAS-B
4	2005	33	Acute myeloid leukemia	2	Hemolytic streptococci group G	Plasma
5	2005	58	Mantle cell lymphoma	2	Bacillus Cereus	PAS-B
6	2005	46	Aplastic anemia	3	Staphylococcus aureus	PAS-B
7	2005	58	Non Hodgkin lymphoma	2	Hemolytic streptococci group G	Plasma
8	2008	53	Acute myeloid leukemia	2	Coagulase negative staphylococci	Plasma
9	2010	72	Prostate carcinoma	1	Coagulase negative staphylococci	PAS-B
10	2010	39	Acute myeloid leukemia	2	Streptococcus dysgalactiae	PAS-B
11	2011	59	Acute myeloid leukemia	2	Salmonella group B	Plasma
12	2012	75	Non Hodgkin lymphoma	2	Hemolytic streptococci group C	Plasma
13	2013	62	Chronic lymphoid leukemia	2	Coagulase negative staphylococci	PAS-C
14	2014	60	Multiple myeloma	4	Staphylococcus aureus	Plasma

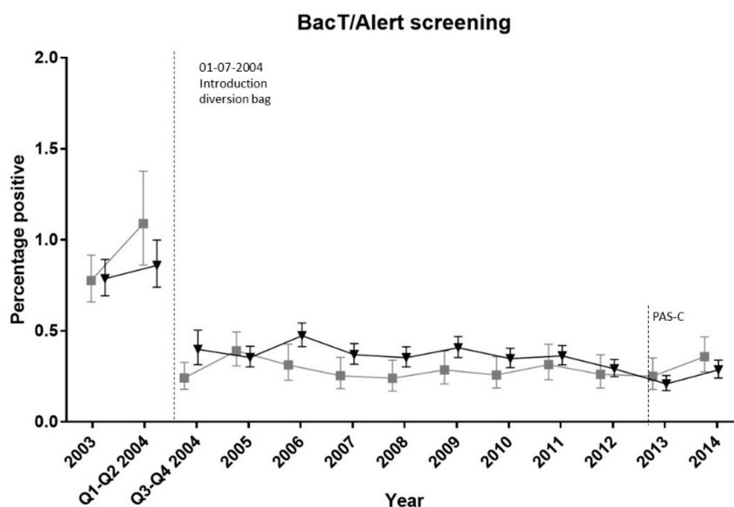
** Severity of transfusion reaction. Grade 1: minor morbidity, not life-threatening; grade 2: Moderate to serious morbidity, may or may not be life-threatening; or leading to hospitalisation or prolongation of illness; or associated with chronic disability or incapacity; grade 3: serious morbidity, directly life-threatening; grade 4: mortality following transfusion reaction.*

†N/A, Not available, information was not reported to TRIP.

The increased risk of TTBI after transfusion of PAS stored platelet concentrates could be explained by auto-sterilisation of plasma stored platelet concentrates, which potentially inhibits a high bacterial load in a contaminated product. The aforementioned *in vitro* studies showed differences in growth characteristics of some bacterial strains suggesting improved sensitivity of bacterial screening of platelet concentrates stored in PAS-C of PAS-E. However, as shown in figure 1, the frequency of confirmed positive results was higher for platelet concentrates stored in plasma compared to those stored in PAS-B. This is in line with the results of a

previous study which compared the screening results of all platelet concentrates in 2002 and 2003.[18] With our data, it was not feasible to compare the different generations of PAS, since PAS-C has only been in use for two years, during which only one case of TTBI related to PAS-C has been reported.

Figure 1. Percentage of confirmed positive results for all screened platelet concentrates screened by storage medium



Confirmed positive means a microorganism could be isolated from the positive bottle.[14] The diversion pouch has bene universally used since 1st July 2004. PAS-C has been in use since 1 January 2013.

This is the first clinical study investigating the association of storage medium of platelet concentrates with TTBI. Storage media differs among countries and several generations of additive solutions are used.[19] Incidences of TTBI could not be compared between countries, due to large differences in hemovigilance.[17]

In the Netherlands the choice of storage medium is determined solely by location of the hospital. Since it is likely that characteristics of patients receiving platelet concentrates are similar in different regions of the Netherlands, we expect that these are also equally distributed among storage media. Because most cases were diagnosed with hematological malignancies, we performed an additional check, selecting only those hospitals licensed for autologous or allogeneic stem cell transplantations. Among these hospitals, 20.4% of platelet concentrates were stored in PAS, which is comparable to the 22.3% observed for all hospitals. This reaffirms our assumption that patient characteristics are similar among the different

regions. Furthermore, differences in vigilance in reporting of TTBI could confound the results. The hospitals in which PAS stored platelet products are used reported 28.1% of TTBIs related to red blood cell products, whereas these hospitals transfused 22.6% of all red blood cell products (RR 1,34 (95% CI: 0,87-2,08)). This seems to indicate that differences in reporting behaviour cannot explain the observed strong association.

A limitation of this approach is that platelet concentrates in PAS and plasma were produced at different blood bank locations. Differences between these locations could theoretically also have affected the risk of TTBI. However, it seems unlikely that this could fully explain the observed strong association of storage medium with risk of TTBI.

To conclude, transfusion of PAS stored platelet concentrates is associated with a four-fold increased incidence of TTBI, compared to plasma stored platelet concentrates.

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Supplemental material

Available at http://onlinelibrary.wiley.com/doi/10.1111/trf.13969/abstract

Table S1. Distribution of storage medium over the years

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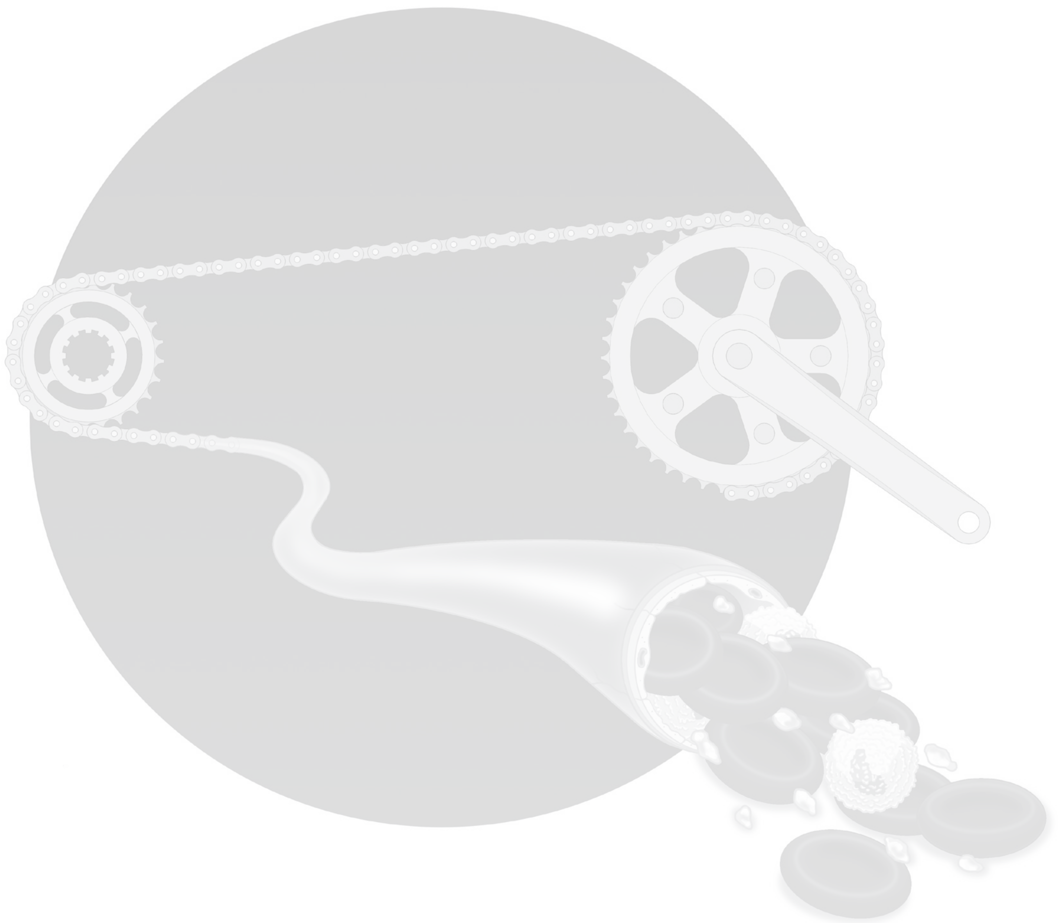


Chapter 10

General discussion

Blood transfusions are one of the most common procedures in hospitals and an essential part of supportive care in the treatment of hematological malignancies.[1] For the research presented in this thesis we used routinely collected health care data to investigate the safety and effectiveness of platelet transfusions in hematological patients.

Routinely collected health care data

Routinely collected health care data constituted the cornerstone of several studies described in this thesis. In general, big data, including routinely collected health care data, are increasingly used in research.[2,3] By using routinely collected health care data, observational studies can reach sample sizes which are 100- to 1000-fold bigger while minimizing costs and effort. This gives the opportunity to study subgroups which are often overlooked in randomized controlled trials or to investigate rare events. Moreover, trials are not always feasible or ethical and patients in trials are selected using stringent in- and exclusion criteria resulting in limited generalizability, whereas patients in this kind of observational studies reflect daily clinical practice.[4,5] However, the use of routinely collected health care data is criticized as these data are not collected with research as the prime motive, but healthcare driven. This could imply that the data is not complete, the level of detail is less than desired, or the information is not uniformly coded.[6-8] Therefore, the investigator has to ensure the completeness, validity, and applicability of the data for the question of interest.

Incompleteness due to underreporting is one potential source of bias which could arise by using this kind of data. For the research presented in **chapter 9**, we used the national register of transfusion reactions as main data source. Reporting of severe transfusion reactions, like transfusion transmitted bacterial infections, is compulsory under European law, which ensures completeness of this register regarding these reactions.[9,10] In **chapter 8**, we used databases of Denmark, to what, for a reason, is referred as 'not a country, but a cohort'.[8] The Danish government underlines the importance of epidemiological research and facilitates the required infrastructure. As a consequence, the entire country is covered and registers can be individually linked via the personal registration number, ensuring complete follow-up.[11,12]

The validity of the data determines the reliability of research. In contrast to laboratory measurements, diagnostic and procedural codes, like DBC codes and ICD codes, are prone to interpretation.[13] Coding is especially inaccurate for poorly defined diseases with a high prevalence, like asthma or diabetes.[14] For the research

presented in this thesis, we used DBC codes to identify hematological patients. Chart review, used as golden standard for the development of the model described in **chapter 4**, revealed that this coding was correct for all patients in the sample. Besides via chart review, validity of the data could also be assessed by comparing the data with other data sources. The validity of ICD codes is, for example, evaluated by linking these data to data of the West of Scotland Coronary Prevention Study (WOSCOPS) trial. The WOSCOPS trial aimed to evaluate the effect of pravastatin on cardiovascular endpoints.[15] Eighty percent of the non-fatal cardiovascular endpoints and even 99% of fatal events could be linked with routinely recorded ICD codes.[16] Thus, although ICD and DBC codes are prone to differences in use and changes in definitions, the validity of the data, with respect to these outcomes, seemed to be good.

The applicability of the data depends upon the depth of the information. The depth may be insufficient when not all information a researcher needs for a specific study is accurately recorded in the registry or database.[8] Proxies could be used to overcome this lack of detailed information. In **chapter 7 and 8**, we used positive blood cultures as a proxy for clinically relevant infections. This automatically implicates a certain degree of misclassification, as not all positive blood cultures are accompanied by clinical symptoms. However, this misclassification is not related to the exposure of interest, in this case storage time of the transfused product, neither to other variables nor to errors in these variables. Therefore, it is most likely that this non differential misclassification will have resulted in bias towards the null and thereby an underestimation of the true effect.[17] The alternative of using a single variable as a proxy, is to combine several variables into a model to predict or identify certain outcomes. In **chapter 4** we described such a model to identify leukemic patients with major hemorrhage based on information regarding CT scan of the brain, drop in hemoglobin level, and need of transfusions.

When the completeness, validity and applicability of the data is ensured, practical hurdles have to be taken before the data can be actually used. The key problem in retrieving the data is that a large amount of data is recorded as a by-product of health care and leverage of the information therein is not straightforward. At first glance, laboratory measurements and transfusion data are the most easily accessible data, as these are not prone to different interpretations. However, hospitals use different computer systems, like GLIMS, LABOSYS, MOLIS, or Labtrain, and even within the same program each hospital could set up its own feature. As a consequence, queries to obtain the data are not interchangeable between hospitals.

In the ATTACH study, we assembled data regarding transfusions, laboratory measurements, microbiology, and DBC codes in nine hospitals. As an ongoing study, most of the gathered data is incorporated into the Dutch Transfusion Datawarehouse, which will be updated regularly.[18] Other examples of such large transfusion databases are the Scandinavian Donation And Transfusion Database 2 (SCANDAT2) which we used in the study described in **chapter 8**, registers in Finland and Canada, or the REDS-III program in the United States.[19-22] In England, the National Health Service Blood and Transplant (NHSBT) planned to develop a transfusion dataset that can be downloaded from the hospitals into a datawarehouse.[23]

So, many efforts have been made to obtain transfusion databases and these will constitute a key element in future transfusion research. In the research described in this thesis, we applied the aforementioned methods to obtain and analyze data from various resources to assess safety and effectiveness of platelet transfusions.

The platelet concentrate: storage medium

As illustrated by the research presented in **chapter 7, 8, and 9**, transfusions are not without side effects and could even deteriorate the clinical situation of a patient. The thrombocytopenia for which hematological patients require platelet transfusions is often accompanied by neutropenia, leading to an increased risk of infections. The storage conditions of platelet concentrates facilitate ideal circumstances for bacterial growth once a product is contaminated.[24] These growth characteristics vary among storage media. Compared to plasma, bacteria initiate the log-phase faster in PAS and after 24 hours the concentration of bacteria is higher although the maximum bacterial concentration is similar in both storage media. In addition, there is less biofilm formation in PAS and this could potentially result in a larger amount of bacteria available for sampling and thereby a lower risk of false negative screening results.[25-27]

The incidence of transfusion transmitted bacterial infections is very low, approximately 22 per million platelet transfusions in the Netherlands. This corresponds to one case each year. Despite the fact that our database encompassed more than a decade, we could include only fourteen cases in the study described in **chapter 9**. Although comparing incidences between countries would result in more cases, this estimate would be confounded by differences in definitions, vigilance, transfusion indications and patient characteristics, which are hard to quantify. The distribution of storage media in the Netherlands provides the unique opportunity to

perform such a study within one country. In the additional analyses we have demonstrated that hemovigilance and patient characteristics were similar over the regions. The risk of transfusion transmitted bacterial infections was a fourfold increased after transfusion of PAS-stored platelet concentrates, although the aforementioned differences in growth characteristics did not result in an increased incidence of confirmed positive screening results. Apparently, the differences in growth characteristics do not result in differences at the moment of screening, but do make a clinical difference after storage. Whether the presence of proteins like complement in plasma contribute to this phenomenon requires further research.
Many attempts are made to further reduce the risk of transfusion associated infections with pathogen reduction technologies. These have the major advantage that it eliminates all kind of pathogens, including bacteria, viruses, and unknown pathogens. However, it could be questioned whether this is cost effective compared to current screening policies.[28] Based on the results of our study, it could be advised to use plasma as storage medium for platelet concentrates to reduce the risk of transfusion transmitted bacterial infections. However, PAS has several other advantages such as a lower risk of other transfusion reactions, like allergic reactions.[29-31]

Besides differences in safety profile, PAS and plasma stored platelet concentrates may also differ in effectiveness. Platelet concentrates stored in PAS-C had lower 1 and 24 hour corrected count increments compared to plasma stored platelet concentrates.[32,33] Newer generations of PAS showed similar *in vitro* quality characteristics as plasma.[29] More important from a clinical and patient's perspective are differences in bleeding rates. The aforementioned studies were not powered sufficiently to assess this outcome. The model described in **chapter 4** could be used to compare effectiveness of platelet concentrates between regions which use PAS or plasma stored platelet concentrates, similar as the approach used in **chapter 9**. However, the endpoint in the latter study, transfusion transmitted bacterial infections, was directly related to a single transfusion. Such a direct association cannot be assumed between transfusion and major hemorrhage. In addition, we described the large variation in clinical practice among hematologists in **chapter 2**. Whereas in one hospital a patient will receive a transfusion before removal of a central venous catheter when the platelet count is below $40x10^9$/L, this patient will receive this transfusion not before the platelet count drops below $10x10^9$/L in another hospital. This variation in daily practice challenges a direct comparison of the effectiveness of platelet concentrates stored in PAS or plasma, but with adequate adjustments for variation in clinical practice, studies based on routinely

collected health care data can be a valid guide in deciding which storage medium should be used and whether newer generations of PAS should be implemented. Nowadays, such decisions are based on the results of *in vitro* studies or trials that were powered on laboratory measurements, which are at most proxies for clinically relevant outcomes. Moreover, before a decision can be made which storage medium should be used, a cost effectiveness analyses should be made to take the stock of all clinical relevant differences in safety and effectiveness.

The platelet concentrate: storage time

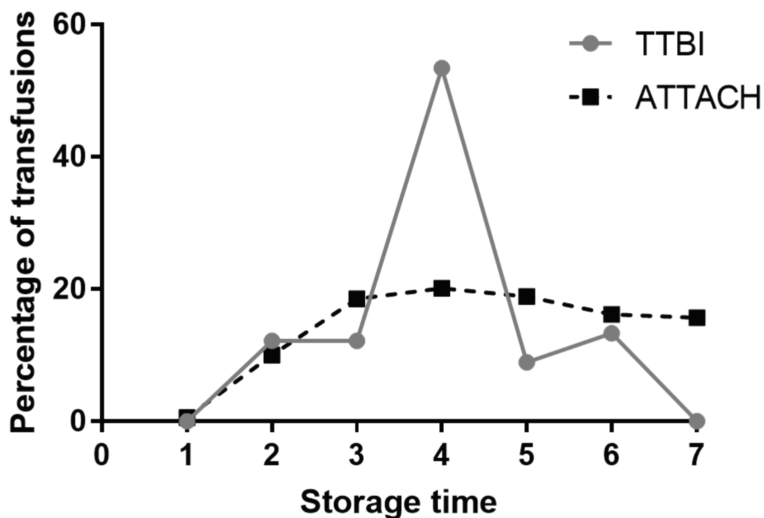
Besides storage medium, also storage time influences safety and effectiveness of platelet transfusions. As shown in **chapter 5 and 6** fresh platelets have better increments and showed superior survival and recovery. In addition, less transfusion reactions occurred after transfusion of fresh, non-leukoreduced platelets. The detrimental effect of storage time on risk of transfusion reactions was not seen when the platelet concentrates were leukoreduced. Hematological patients need more platelet transfusions when older products are transfused as the interval between transfusions is shortened and the risk of bleeding may increase with increasing storage time.

Although fresh platelets seems superior regarding several measures of effectiveness, safety concerns, especially bacterial infections, remain the main reason to restrict maximal storage time. This highly varies between countries, ranging from 3.5 days without bacterial screening to 5 or 7 days with the implementation of universal bacterial screening.[34] In March 2016, the FDA published a draft guideline in which they announced extension of maximum storage time up to seven days, provided that all products are screened prior to transfusion. However, up to date, no screening method has been certified as an adequate safety measure.[35]

The assumed increased risk of transfusion transmitted bacterial infections is based on several case reports of severe septic reactions after transfusion of platelet concentrates stored for four days or more.[36-39] In **chapter 9**, we specifically studied these adverse transfusion reactions. Storage time is recorded for eleven of the fourteen cases with transfusion transmitted bacterial infections. The median storage time of the products involved in these reactions was 4 days (IQR 4 to 5.5), compared to 5 days (IQR 3 to 6) for all products in the ATTACH study. However, storage time for products stored in PAS-B, which was used up to 2012, was restricted to 5 days and whereas 56.3% of the products involved in a TTBI was stored in PAS,

only 26.7% of the products in the ATTACH study were stored in PAS. Figure 1 shows the directly standardized storage time of products involved in TTBIs, compared to the storage time of all transfused products between 2005 and 2014 in the ATTACH study. This indicates that restriction of storage time to 5 days does not reduce the risk of TTBI and that safety concerns seems no valid reason to limit storage time to 5 days, under the condition that all products are screened by the BacT/Alert.

Figure 1. Storage time of transfused products involved in TTBI and the ATTACH study



In contrast to this assumed increased risk during storage, we showed in **chapter 7 and 8** that storage time was not associated with an increased risk of all-cause bacteremia. If anything, the risk even decreased with increasing storage time. In both studies, we used a positive blood culture of the recipients as a proxy for bacterial infections. Inherent to such a strategy is that we grouped transfusion transmitted bacterial infections with bacterial infections of all other causes. Rationale for this approach was that bacterial infections could be caused directly by the transfusion, but also indirectly via modulation of the immune system.

Transfusions could have an immunosuppressive effect, which is beneficial in transplantations and autoimmune diseases, but could be detrimental in oncological diseases and infections. Most research regarding transfusion related

immunomodulation focuses on red blood cell transfusions.[40-42] It has been speculated that the immunomodulatory effect of red blood cell transfusions could be attributed to the remaining platelets or plasma in the product.[40,43] In critically ill patients, neither red blood cell transfusions, nor plasma transfusions were associated with an increased risk of nosocomial infections, whereas platelet transfusions were identified as an independent risk factor.[44] It has been hypothesized that platelets not only play a role in hemostasis, but also have immunological capacity.[45,46] This theory is supported by the expression of HLA class I molecules and the ability to secrete mediators.[43,47] During storage, platelets lose the expression of HLA class I molecules and thereby the ability to stimulate antibody production. Moreover, only fresh platelets were able to modulate skin graft rejection in mice.[47] The potential immunomodulatory effect of fresh platelet may explain our findings of a lower risk of all-cause bacteremia after transfusions of older platelet concentrates. However, this remains speculation and the pathogenic mechanism explaining our findings has to be entangled.

The patient

Besides all aspects of the products, transfusing at the moment the patients benefit the most from it, remains the fundamental key of good practice. For hematological patients, the moment when to transfuse platelets seems clearly specified in the guidelines: prophylactically when the platelet count drops below $10x10^9$/L or therapeutically in case of bleeding.[48-50] However, recommendations are lacking for patients who may face an increased risk of bleeding or need an invasive procedure. The results of the survey described in **chapter 2** indicated a large variation in clinical practice, suggesting over-, as well as under-treatment of certain patients. In order to improve supportive care, risk factors of bleeding need to be identified and we need to know to which extent platelet transfusions are able to reduce this risk to enable the development of a personalized transfusion threshold for each situation.

As demonstrated in **chapter 3**, transfusions are not effective in all patients. Patients who have developed multiple HLA-alloantibodies require platelet concentrates from HLA matched donors. However, HLA highly varies among ethnicities and blood banks face the major challenge to find suitable donors for all immunized patients in the current multicultural society with mixing of cultures. Lack of an acceptable donor could even force physicians to refrain from treatment, as no adequate support can be supplied. Selective HLA typing of donors from all required ethnic backgrounds would increase the variation in HLA phenotypes in the current HLA-typed donor

population and enhance the availability of HLA matched platelet products for non-Caucasian, immunized patients.

The future

With the studies presented in this thesis we assessed the safety and effectiveness of platelet transfusions by using routinely collected health care data. Transfusion thresholds in specific situations and identification of risk factors for bleeding have not received much attention so far. Several challenges have to conquered to ensure the use of routinely collected health care data in the future to study these topics. The first issues which have to be addressed are the privacy of the patient, informed consent, confidentiality, security, and ownership of the data.[51-53] These are subject of an ongoing discussion and National and European laws and guidelines are changing. Obtaining informed consent from a large amount of participants can pose a financial and bureaucratic burden for research and when informed consent is routinely asked from all patients in a hospital, the 'informed' part of the consent may be violated. Not all possible studies are known at the moment the data is collected, which makes it impossible to fully inform patients about all future uses of the data. When historical data are used, asking informed consent can be a disproportional burden and invasion of personal life. It has been argued that explicit informed consent is not required for database research when data can be anonymized or analyzed at a group level. So, it remains a delicate balance between ethical ideals of data protection and informed consent on one hand and the use of gathered data for medical research on the other hand.

Within databases of routinely collected health care data, detailed information about signs and symptoms of the patient and considerations of the threating physician is lacking. This constitutes probably the most valuable part of information, but also the most challenging part to unravel. Manual review of medical charts is labor intensive and hampers the ability to examine large numbers of patients in an efficient manner. Natural language processing can automatically interpret this information and makes it available for analyses. It has been used for example for the identification of postoperative complications, but many investments are still needed to make it suitable for the analysis of all unstructured medical notes.[54,55] This wouldn't be necessary if registration is in such a way that data are also applicable for research purposes. A good initiative to promote this, is 'Registratie aan de bron', a program from the Nederlandse Federatie van Universitaire Medische Centra (NFU) and Nictiz to stimulate unambiguous registration and facilitate transmission of data between hospitals for research, bench marking, and quality control.[56,57]

To conclude, a close collaboration of researchers, clinicians, and ICT is needed to develop a digital system which does not interfere, but supports daily practice, improves efficiency, and enables optimal use of all recorded data. In addition, clinical knowledge and a strong epidemiological foundation are indispensable to convert the immense potential of big data into valuable, clinically relevant, research results.[12,58,59] When these requirements are met, studies regarding safety and efficacy of blood transfusion can focus on clinically relevant outcomes, reflect daily practice which will amplify generalizability, and in the end improve and personalize supportive care for all future patients.

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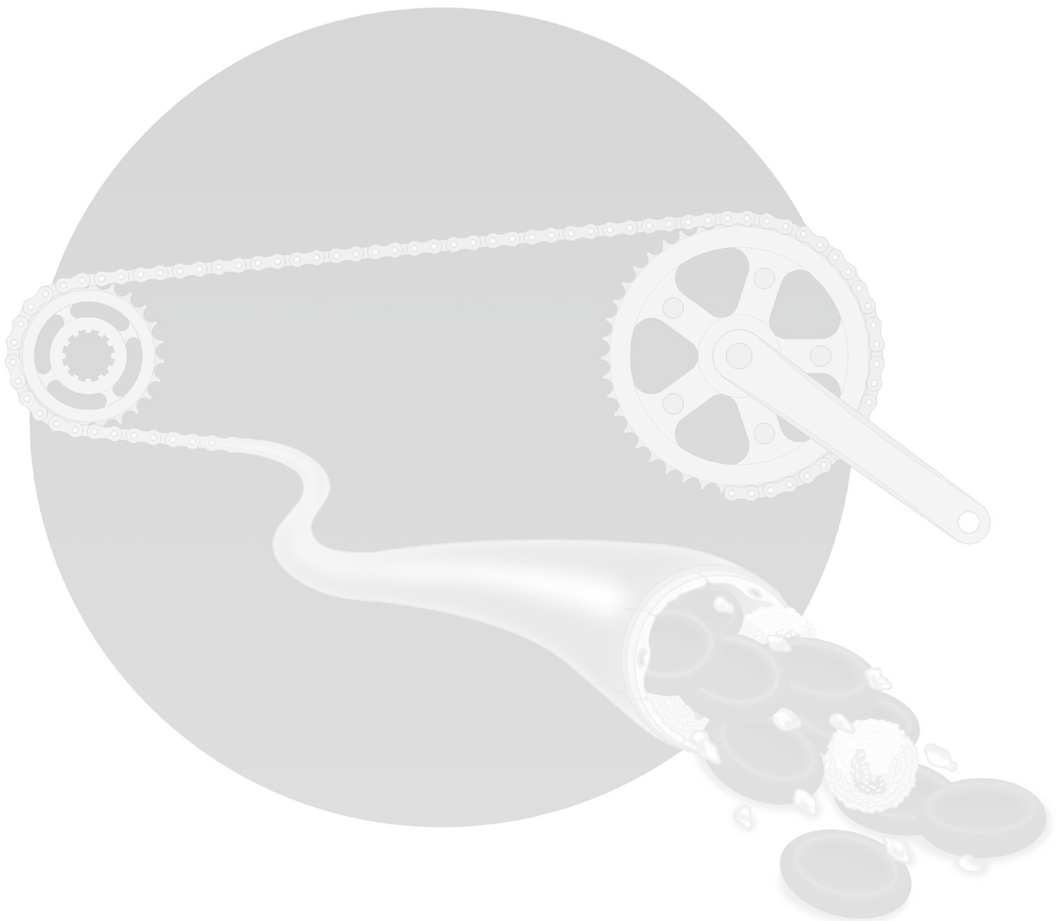


Chapter 11

Summary
Nederlandse samenvatting

Summary

The majority of platelet transfusions are given to patients with a hematological malignancy to prevent or treat bleeding complications. Although the recommendations are clear for clinically stable patients, less evidence exists for patients who face an increased risk of bleeding or need an invasive intervention. This results in a large variation in daily practice as we demonstrated with a survey among Dutch and European hematologists.

In the Netherlands, the standard platelet concentrate is derived from buffy coats of five AB0-identical donors and resuspended in plasma of one of these donors or platelet additive solution (PAS). Platelets express HLA class I antigens, and alloantibodies against common HLA antigens may cause accelerated destruction of transfused platelets, resulting in lower increments. Our evaluation of the current policy for refractory patients in the Netherlands, showed that these patients benefit the most from HLA split matched platelet concentrates. Although almost 20.000 donors are HLA typed, adequate transfusion support cannot be guaranteed for all refractory patients. Increased heterogeneity within the donor population is warranted to ensure sufficient support for immunized patients from a non-Caucasian background.

Despite major improvements in the production process and storage conditions of platelet concentrates, major hemorrhages and transfusions reactions have not been completely eliminated. The incidence of these adverse events is low, so large sample sizes are required to obtain sufficient power to investigate the safety and effectiveness of transfusions. We used routinely collected health care data to study these rare outcomes.

From a clinical perspective, major hemorrhage is the most relevant outcome to measure effectiveness of platelet transfusions. However, hemorrhages are not uniformly coded and proxies are needed to detect patients with major hemorrhage in large databases. We developed a model consisting of drop in hemoglobin, transfusion support, and CT-brain to enable the identification of major hemorrhage among leukemic patients in such databases.

Platelet concentrates are stored in a gas permeable bag, under constant agitation at room temperature, for a maximum of seven days. During storage, platelets get activated and show a gradual loss of function *in vitro.* In a meta-analysis, we showed that older platelets are associated with lower increments, and inferior survival and recovery after transfusion. Moreover, the interval between transfusions of older

platelet concentrates is shortened compared to fresh platelet concentrates and as a consequence, hematological patients need more transfusions. The risk of bleeding may be increased. When platelet concentrates are leukoreduced, storage time does not affect the risk of transfusion reactions.

Platelet concentrates carry the highest risk of infections compared to red blood cell or plasma transfusions due to the storage at room temperature. The assumed increased risk of transfusion transmitted bacterial infections with increasing storage time is the main argument to shorten the maximum half-life of platelet concentrates in several countries. In the ATTACH study, we assembled routinely collected health care data of nine hospitals spread around the Netherlands to investigate the association of storage time with the risk of bacteremia after transfusion in hematological patients. Platelet concentrates stored in plasma for five to seven days were associated with a lower risk of bacteremia. Similarly, we showed a lower risk of a positive blood culture after transfusion of older platelet concentrates stored in PAS in all recipients of platelet transfusions in Denmark. Therefore, we used SCANDAT, a binational transfusion database of Denmark and Sweden, combined with MiBa, the Danish microbiology database. In these studies, we used a positive blood culture as proxy for a bacterial infection and these are not necessarily causally related to the transfused blood product. When the same microorganism is identified in the transfused product and in the patient, the infection is classified as a Transfusion Transmitted Bacterial Infection and these have to be reported to TRIP (Transfusion and Transplantation Reactions In Patients), the Dutch competent authority of hemovigilance. Storage medium influences bacterial growth characteristics in the product. In the Netherlands, platelet concentrates stored in plasma as well as in PAS are concurrently used and the geographic location of the hospital determines which storage medium is used. Using the database of TRIP, we showed an increased risk of transfusion transmitted bacterial infections for platelet concentrates stored in PAS compared to those stored in plasma.

Nederlandse samenvatting

Het bloed van een gezond individu bevat ongeveer 150-400x10^9 trombocyten per liter. Bij patiënten met een hematologisch maligniteit kan dit dalen tot onmeetbaar lage waarden. In 1910 beschreef Duke als een van de eersten de mogelijke rol van transfusie van trombocyten bij het stoppen van bloedingen. Gaydos et al beschreven in 1962 de onmiskenbare relatie tussen een laag trombocyten getal en het risico op bloedingen en deze studie wordt gezien als grondlegger van een profylactisch transfusiebeleid. Tegenwoordig zijn profylactische trombocytentransfusies een essentieel onderdeel van de ondersteunende behandeling bij hematologische maligniteiten.

Ieder jaar worden in Nederland ongeveer 59.000 trombocytenconcentraten getransfundeerd, waarvan het merendeel naar hemato-oncologische patiënten gaat. Het onderzoek beschreven in dit proefschrift beoogt de veiligheid en effectiviteit van trombocytentransfusies bij hemato-oncologische patiënten te verbeteren.

Als het gaat om een stabiele patiënt is er in de kliniek weinig discussie omtrent het te volgen transfusiebeleid en wordt over het algemeen een streefwaarde van minimaal 10x10^9 trombocyten per liter aangehouden. Bij patiënten met bijkomende problemen, zoals eerder doorgemaakte bloedingen, actieve infecties, het gebruik van medicatie, of voor een invasieve ingreep, lijkt het verdedigbaar om een hoger trombocytenaantal na te streven. Bewijs hiervoor ontbreekt echter en internationale richtlijnen zijn niet eenduidig. Zo wordt voor een lumbaalpunctie een transfusiedrempel van 20x10^9/L geadviseerd in de Nederlandse CBO-richtlijn, terwijl een drempel van 50x10^9/L geadviseerd wordt in de Britse richtlijn. Dit resulteert in grote variatie in transfusiebeleid, welke wij in kaart brachten met een vragenlijstonderzoek onder Nederlandse en Europese hematologen. In de praktijk varieerde het nagestreefde trombocytengetal voor een lumbaalpunctie tussen de 10 en 100x10^9/L. Eenzelfde grote variatie in richtlijnen en beleid werd gezien bij het verwijderen van een centraal veneuze lijn.

Bij een klein deel van de getransfundeerde patiënten is de opbrengst van een trombocytentransfusie laag. Een van de mogelijke oorzaken is de vorming van antistoffen tegen HLA klasse I antigenen die op trombocyten tot expressie worden gebracht. Voor deze patiënten kunnen trombocyten van een HLA gematchte donor uitkomst bieden. In Nederland zijn ongeveer 20.000 donoren voor dit doel getypeerd, maar desondanks zijn er voor ongeveer 10% van de geïmmuniseerde

patiënten niet voldoende donoren beschikbaar om adequate support te kunnen bieden bij de behandeling van bijvoorbeeld acute leukemie. Dit wordt onder andere veroorzaakt door verschillen in etnische achtergrond tussen patiënten en donoren.

Trombocytentransfusies zijn primair geïndiceerd ter preventie of behandeling van bloedingen. Bij het evalueren van de effectiviteit van trombocytentransfusies zijn bloedingen dan ook het meest relevante eindpunt. Tevens is het van belang om risicofactoren voor bloedingen te identificeren om de transfusiedrempel te kunnen personaliseren en onnodige transfusies te voorkomen. Door de lage incidentie van ernstige bloedingen zijn grote patiëntengroepen vereist om valide uitspraken te kunnen doen. Gegevens die routinematig worden bijgehouden in de dagelijkse praktijk maken observationeel onderzoek in grote populaties mogelijk, maar het optreden van ernstige bloedingen wordt niet op een gestandaardiseerde manier geregistreerd. Derhalve hebben wij een model ontwikkeld om leukemie patiënten met ernstige bloedingen te identificeren in routinematig verzamelde data aan de hand een daling van het hemoglobine, het aantal transfusies in 24 uur en het al dan niet verrichten van een CT-scan van de hersenen. Met behulp van dit model kan toekomstig onderzoek naar bijvoorbeeld risicofactoren voor bloedingen op een efficiëntere manier verricht worden.

Trombocyten worden bewaard in plasma of Platelet Additive Solution (PAS), een gestandaardiseerde elektrolytensamenstelling. In PAS bewaarde trombocyten worden gebruikt in ziekenhuizen in Zuid-West Nederland en in de overige regio's worden in plasma bewaarde trombocyten gebruikt. In de circulatie overleven trombocyten tien dagen, dus daaruit volgend is de gemiddelde leeftijd van gedoneerde trombocyten ten tijde van de donatie ongeveer vijf dagen. Door het optimaliseren van de bewaarcondities is het mogelijk om trombocytenconcentraten tot zeven dagen na afname in goede conditie te houden. Het verouderen heeft echter wel een effect op de veiligheid en effectiviteit van de transfusie.

Transfusies van langer bewaarde, dus oudere, trombocyten hebben een lagere opbrengst en kortere overleving. Daarnaast is het interval tussen twee opeenvolgende transfusies korter wat resulteert in een toegenomen transfusiebehoefte bij hematologische patiënten. Mogelijk gaat langer bewaren tevens gepaard met een verhoogd risico op bloedingen. Bij niet-leukogereduceerde trombocytenconcentraten werd eveneens een verhoogd risico op transfusiereacties gezien bij oudere producten.

Trombocyten worden bewaard op kamertemperatuur in een gasdoorlaatbare zak terwijl ze constant geschud worden. Dit zijn ideale omstandigheden voor proliferatie van bacteriën en daarom worden in Nederland alle trombocytenconcentraten direct na productie gescreend op bacteriële contaminatie met het BacT/Alert systeem. Desondanks is het risico op transmissie van bacteriële infecties bij trombocytentransfusies groter dan bij andere bloedproducten. Voor sommige landen is dit de reden om de maximale bewaarduur te beperken. Zonder bacteriële screening is de maximale bewaarduur slechts drie-en-een-halve dag in Japan en vier dagen in Duitsland, terwijl dit met screening beperkt is tot vijf dagen in de Verenigde Staten en Frankrijk. In onder andere Nederland en Denemarken worden trombocytenconcentraten maximaal zeven dagen bewaard. Aangezien trombocyten naast een rol in de hemostase ook immunologische capaciteiten bezitten, is ook immunomodulatie gesuggereerd als mechanisme dat na een transfusie tot infecties kan leiden.

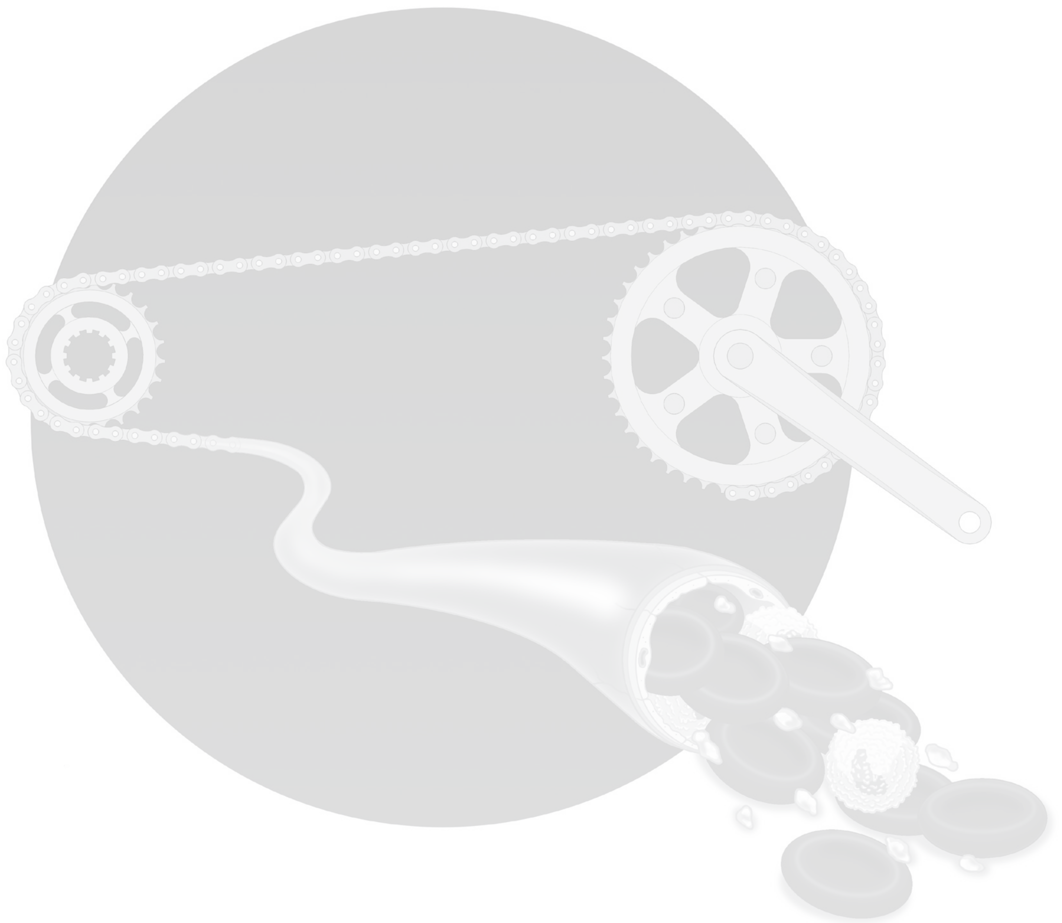
In de ATTACH studie hebben wij het effect van bewaarduur op het risico van een bacteriemie onderzocht bij hematologische patiënten. Hiervoor hebben wij routinematig verzamelde gegevens van negen ziekenhuizen in Nederland gecombineerd met data van Sanquin met betrekking tot de karakteristieken van getransfundeerde bloedproducten. Het risico op een bacteriemie bleek niet toe te nemen met de bewaarduur en transfusie van trombocyten die vijf tot zeven dagen bewaard waren in plasma bleek zelfs geassocieerd te zijn met een lager risico op een bacteriemie dan wanneer de trombocytenconcentraten een of twee dagen oud waren. Het aantal producten bewaard in PAS was te laag om een dergelijk effect ook voor dit bewaarmedium aan te kunnen tonen.

In Denemarken worden enkel in PAS bewaarde trombocyten gebruikt en data omtrent alle transfusies in Denemarken en Zweden worden verzameld in de Scandinavian Donation And Transfusion (SCANDAT2) database. De gegevens van de Deense patiënten hebben wij gekoppeld aan de Deense microbiologie database (MiBa). In lijn met de resultaten uit de ATTACH studie bleek het risico op een positieve bloedkweek de dag na transfusie van een trombocytenconcentraat van zes of zeven dagen oud lager dan na een trombocytenconcentraat van een tot vijf dagen oud. Dit effect gold voor alle patiënten die een trombocytentransfusie ontvingen.

In zowel de ATTACH studie als de Deense studie hebben wij een positieve bloedkweek de dag na transfusie gebruikt als proxy voor een bacteriële infectie. De restanten van de getransfundeerde producten zelf zijn niet meer gekweekt, dus met deze studieopzet kan niet aangetoond worden dat contaminatie van het product

een rol heeft gespeeld bij het optreden van de infectie. Wanneer hetzelfde micro-organisme zowel in de patiënt als in het bloedproduct wordt gekweekt, wordt dit gedefinieerd als een transfusie-transmissie bacteriële infectie en het is wettelijk verplicht om deze reacties te rapporteren bij TRIP, het nationaal bureau voor hemovigilantie en biovigilantie. Elk jaar krijgt ongeveer 1 patiënt een sepsis na transfusie van een gecontamineerd trombocytenconcentraat. Het risico op een dergelijke infectie is groter wanneer de trombocyten bewaard worden in PAS dan in plasma.

Voor het onderzoek beschreven in dit proefschrift hebben wij gebruik gemaakt van routinematig verzamelde gegevens uit de dagelijkse patiëntenzorg. Door de lage incidentie van zowel ernstige bloedingen als transfusiereacties is onderzoek in grote populaties noodzakelijk om valide uitspraken te kunnen doen over de effectiviteit en veiligheid van trombocytentransfusies. Het onderzoek in dit proefschrift toont aan dat routinematig verzamelde patiëntgegevens hier uitkomst in kunnen bieden. Verbetering en uniformering van de huidige systemen is echter noodzakelijk om alle gegevens die in de dagelijkse patiëntenzorg worden verzameld optimaal te kunnen benutten voor wetenschappelijk onderzoek.



Chapter 12

Curriculum vitae
List of publications
Dankwoord

Curriculum Vitae

Aukje Lydia Kreuger werd geboren op 18 juni 1988 in De Bilt. In 2006 behaalde zij cum laude haar gymnasium diploma aan het Kalsbeek college te Woerden, waarna zij begon aan haar studie Geneeskunde aan de Universiteit van Leiden. Tijdens haar studie deed zij onder andere onderzoek op het laboratorium van de Klinische Oncologie naar immunologische factoren die het effect van adoptieve T cel therapie bij melanoom patiënten beïnvloedden. Zij rondde haar studie af met een semi-arts stage op de afdeling Interne Geneeskunde in het Groene Hart Ziekenhuis in Gouda. Na haar artsexamen in december 2013 startte zij met haar promotieonderzoek onder begeleiding van prof. Anske van der Bom, dr. Jean Louis Kerkhoffs en dr. Rutger Middelburg aan het Center for Clinical Transfusion Research, Sanquin, en de afdeling Klinische Epidemiologie van het LUMC. Haar onderzoek richtte zich op de veiligheid en effectiviteit van trombocytentransfusies bij patiënten met een hematologische maligniteit. In het kader van de ATTACH studie verzamelde zij data in verschillende ziekenhuizen in Nederland en tevens heeft zij een samenwerking opgezet met onderzoekers van de SCANDAT2 database in Denemarken. Hieruit voorvloeiend deed zij 2 maanden onderzoek aan het Statens Serum Institut in Kopenhagen. De resultaten van haar onderzoek zijn beschreven in dit proefschrift en heeft zij gepresenteerd op diverse nationale en internationale congressen. Zij heeft haar promotieonderzoek gecombineerd met de opleiding tot epidemioloog B. Sinds januari 2018 is zij werkzaam als ANIOS Interne Geneeskunde in het Hagaziekenhuis in Den Haag.

List of publications

In this thesis

Kreuger AL, Middelburg RA, Beckers EAM,. The identification of cases of major hemorrhage in patients with acute leukemia using routinely recorded healthcare data. *Accepted for publication in Plos One*

Kreuger AL, Rostgaard K, Middelburg RA, Kerkhoffs JH, Edgren G, Erikstrup C, Pedersen OB, Titlestad K, Nielsen KR, Ostrowski SR, Voldstedlund M, van der Bom JG, Ullum H, Hjalgrim H. Storage time of platelet concentrates and risk of a positive blood culture; A nationwide cohort study. Transfusion. 2018; 58(1):16-24.

Kreuger AL, Middelburg RA, Bank CMC, Beckers EAM, van Gammeren AJ, Leyte A, Rondeel JMM, de Vooght KMK, Weerkamp F, Zwaginga JJ, Kerkhoffs JLH, van der Bom JG. Storage time of platelet concentrates and all-cause bacteremia in hematological patients. Transfusion. 2017; 57(9):2096-2103

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** authors contributed equally to this study.*

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Dankwoord

Het resultaat dat hier ligt, is te danken aan de inspanning van velen. Wetenschappelijk onderzoek is een echte teamsport en dat hele team wil ik graag vanaf deze plaats bedanken.

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Alle deelnemers van de ATTACH studie, hartelijk dank voor al jullie hulp en enthousiasme. Karen de Vooght (UMCU), Erik Beckers (MUMC), Adriaan van Gammeren (Amphia), Anja Leyte (AMC), Jan Rondeel (Isala), Cock Bank (ADRZ), Floor Weerkamp (Maasstad), Jo van Wiersum en Martin Schipperus (TRIP), het was een genoegen om met jullie samen te werken. Peter, bedankt voor al je hulp bij het verzamelen en koppelen van de data.

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