Chapter 3

A Multicenter Randomized Study of the Efficacy of Transfusions with Platelets stored in Platelet Additive Solution II versus Plasma

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ABSTRACT

Randomized studies testing the clinical efficacy of platelet additive solutions (PASs) for storage of platelets are scarce and often biased by patient selection. We conducted a multicenter, randomized study to investigate clinical efficacy of platelets stored in PAS II versus plasma, also including patients with clinical complications associated with increased platelet consumption. A total number of 168 evaluable patients received pooled buffy coat derived platelet concentrates (PC) suspended in either plasma (n = 354) or PAS II (n = 411), stored up to 5 days. Both univariate as well as multivariate analysis showed a significant effect of used storage medium in regard to 1- and 24 hour count increments and corrected count increments, in favour of plasma PCs. However, there were no significant differences between the groups regarding bleeding complications and transfusion interval. Adverse transfusion reactions occurred significantly less after transfusions with PAS II PCs ($p = 0.04$). Multivariate analysis showed no significant effect of the used storage medium on the incidence of 1- and 24-hour transfusion failure. We showed safety and efficacy of PAS II PCs in intensively treated patients, however plasma PCs show superior increments.

INTRODUCTION

The use of platelet concentrates (PC) for the prevention and treatment of bleeding complications in patients with thrombocytopenia, due to cytotoxic therapy or malignancies of the bone marrow, is generally accepted. Despite the use of prophylactic platelet transfusions, bleeding is a frequent complication and recommendations regarding the preferred transfusion regimen, the quantity and quality of transfused platelets and strategies to monitor efficacy differ and only a minority is evidence based.[1, 2]
In recent decades storage of platelets suspended in non-plasma media (additive solutions) evolved as a growing field of interest. Possible advantages of using additive solutions instead of plasma are an increase of plasma available for plasma products, a reduction of plasma related adverse reactions, improvement of storage conditions in order to increase the shelf-life of PCs and allowing photochemical pathogen reduction techniques.
In the Netherlands the National Blood Supply aims for harmonization of blood products used throughout the country. Currently, except when selected donors are required, all platelet products are prepared using the buffy coat (BC) method. On historical grounds two platelet products are used: Plasma stored platelet concentrates (Plasma PC) and platelet concentrates stored in Platelet Additive Solution II (PAS II PC, Trombosol, Baxter). However, there are no informative studies for a strong selection for one of these products. Although in vitro studies showed significant differences suggesting inferior quality in metabolic, functional and flowcytometric parameters in platelets stored in PAS-II as compared to plasma, platelets stored up to five days in PAS II stay within the range of minimal quality requirements.[3-6] The correlation of these in vitro parameters with clinical efficacy is inconsistent.[7-9] One paired radiolabelled platelet survival study showed a significant decrease in both recovery as well as survival of PAS II PCs compared to plasma PCs and PCs stored in PlasmaLyte A.[10] Data regarding the clinical transfusion response of platelets stored in PAS II are also limited. A small, non-randomized clinical trial did not show a significant difference between PAS II PCs and plasma PCs, and one small prospective, randomized study reported that corrected count increments (CCIs) after transfusion with PAS II PCs were significantly lower.[11, 12] Despite lower CCIs bleeding complications did not differ and the latter study reported a significant reduction in transfusion reactions.[11] Observational analysis of the transfusion response of PAS II PCs and plasma PCs, used in the control arm of a randomized trial evaluating pathogen inactivated platelets (EuroSPRITE), did not show significant differences.[13, 14] Major drawback of these studies was the exclusion of patients with clinical factors known to increase platelet consumption.[12-14] Because several studies show the importance of patient related factors in relation to platelet transfusion response we performed a randomized, controlled, double-blinded study to evaluate the therapeutic efficacy and safety of PAS II PCs in a non-selected patient population.[15-19]

PATIENTS AND METHODS

Patients and study design

The study protocol was approved by the hospital ethics committees and conducted according to the Guidelines of Good Clinical Practice. All patients > 18 year, who needed or were expected to need more than 2 platelet transfusions, were eligible. After informed consent patients were randomized to receive PAS II PCs or plasma PCs. Patients with HLA- and/or HPA-alloantibodies, active immune thrombocytopenia or an indication for CMV-negative blood products (CMV negative patients receiving stem cells of CMV negative, unrelated or HLA-mismatched donors) were excluded. Patients were enrolled at the Hematology departments of two hospitals. Inclusion was restricted to a period of maximal 30 days after the first PC transfusion or a maximum of 8 PC transfusions, whichever occurred first. The inclusion period ended in case of informed consent withdrawal, the occurrence of immunological refractoriness, after request of the patient or the treating physician, or in case of reaching 30 days after the first PC or 8 PC transfusions. In case of the latter two a second randomization was allowed. After randomization age, gender, length, weight, diagnosis, intended treatment, existence of an enlarged spleen (by physical exam and/or imaging techniques), medical history, transfusion history and medication were recorded. Blood samples were tested for ABO-RhD blood group, irregular red blood cell antibodies, hemoglobin, hematocrite, white blood count, platelets, HLA- and HPA-alloantibodies and anti-platelet autoantibodies. During the inclusion period platelet and red cell transfusions, transfusion-related adverse reactions (skin reactions, fever > 2˚C, dyspnoea, hypotension), bleeding complications, mucosal damage, fever, infections, and used medication were recorded. Bleeding complications were graded according to the World Health Organization criteria and mucosal damage was graded according to the Common Toxicity Criteria (version 2.0).[20, 21] Both parameters were reviewed on a daily basis. Infections were scored positive in case of positive cultures or if a focus was likely as shown by radiologic examination.

Platelet concentrates (PCs)

PCs were prepared from five-pooled whole blood BCs with the same ABO blood group.[22, 23] After collection of a unit of whole blood, BCs were prepared through high-speed centrifugation. Five BCs together with one unit of PAS II or one unit of plasma from one of the BC donors were coupled to a BC pool set (containing a transfer bag, leukocyte filter and a PL-2410 storage container, Baxter) through a sterile connection device. After connecting, the five BCs were pooled in the transfer bag together with the unit of PAS II or plasma. A low speed differential centrifugation was used to separate the platelet rich supernatant from erythrocytes and leucocytes, which subsequently was pressed through the leukocyte-filter into the storage container. A sample was obtained prior to storage to measure platelet content, pH and bacterial culture. The platelet content was measured using a Beckman Coulter Act-10 (Coulter Corp., Miami Florida, USA). The PCs were stored at 20-24˚C on a flatbed shaker up to 5 days. The PCs were γ irradiated with 25 Gy at time of issue in case of specific patient requirements for the prevention of transfusion-associated graft-versus-host disease.

Platelet transfusions and monitoring

The treating physician ordered platelet transfusions according to local hospital guidelines. In general, indications were divided in prophylactic trigger-based transfusions, prophylaxis prior to an intervention or treatment of bleeding complications. The transfusion trigger for uncomplicated prophylaxis was ≤ 10 x 10^9/l. In case of serious infections, anti-coagulant medication or administration of anti-thymocyte globulin (ATG) a trigger of ≤ 30 x 10^9/l was used. In case of surgical interventions or bleeding complications a platelet trigger of ≥ 50 x 10^9/l was used. Pre transfusion platelet count was measured one hour prior to transfusion. Platelet counts were measured from 10 minutes to 2 hours after transfusion and from 16 to 24 hours after transfusion to determine the 1- and 24-hour increment, respectively. Platelet counts in the participating hospitals were measured using a Sysmex XE-2100 (Sysmex Corp., Kobe, Japan). In case of a second PC transfusion within 4 hours, both transfusions were considered to be part of one transfusion. If the 24-hour increment exceeded the 1-hour increment, combined with other signs of haematopoietic recovery, then the 24-hour increment value was excluded from analysis. PC transfusion failure was defined as a 1-hour CCI < 7.5 and/or a 24-hour CCI < 4.5. Patients experiencing repeated episodes (≥ 2 subsequent PC transfusions) of PC transfusion failure, without an apparent non-immunological cause were tested for the existence of HLA- and/or HPA-antibodies. If available, PCs of ABO-identical donors were used, although both minor ABO incompatible (i.e. potential donor anti-A and/or B antibodies directed to the platelets/red cells of the patient) and major ABO incompatible PCs (i.e. potential patient anti-A and/or B antibodies directed to the donor platelets) were not excluded.

Study endpoints

The primary endpoints of the study protocol were the 1- and 24-hour CCI, calculated as follows: $CCI_{1/24\,h}$ = [(post transfusion count (x 10^9 /l) $_{1/24\,h}$ – pre transfusion count (x 10^9 /l)) x Body surface area (m^2)]/ Platelet dose (x 10^{11}). Secondary endpoints were transfusion interval, transfusion-related adverse reactions and bleeding complications.
The transfusion interval was defined as the calculated administrative time of two consecutive PC transfusions.

Statistical methods

The study was designed as a two-armed non-inferiority study. The sample size calculation was based on data of patients enrolled in the two randomized trials concerning non-plasma storage media.[12, 13] The standard deviation of the mean 1- and 24-hour CCI was estimated as 6.0. To detect a difference of 30% between the 1- and 24-hour CCI of PAS II PCs and plasma PCs using a 0.05 level 2-sided test, a sample size of 360 transfusions in each study arm provided a power of 90%. The statistical comparison of the CCIs of the two products was performed both as independent transfusion events as well as in a mixed linear model, assuming biological interdependence of consecutive PC transfusions in a patient (SPSS/PC+, Chicago, IL). Fisher-exact tests were used to compare patient characteristics. A multivariate analysis testing the effects on both count increments and CCIs as well as the occurrence of transfusion failure was performed including storage time, storage medium, gender, age, bodyweight, body surface area, diagnosis, therapy, history of prior platelet transfusions, fever (body temperature > 38˚C) at the time of transfusion, infection, splenomegaly and ATG, using a random effects logistic regression model (EGRET).

RESULTS

Patient population

Between October 2003 and April 2005, 195 patients were randomized (plasma PC n = 95; PAS II PC n = 100). A total of 11 patients were excluded (plasma PC n = 7; PAS II PC n = 4) of which 6 patients had HLA-alloantibodies and 1 patient had HPA-alloantibodies prior to the first transfusion, 1 patient developed refractoriness with proven HLA-alloantibodies after the second transfusion, 2 patients acquired an indication for CMV-negative blood products and 1 patient was transferred to another department. Although patients were randomized based on expected platelet transfusions, 16 patients did not receive any platelet transfusion during the inclusion period (plasma PC n = 4, PAS II PC n = 12), resulting in 168 patients, in which platelet transfusions could be evaluated (plasma PC n = 84; PAS II PC n = 84). There were no significant differences between the two study arms (i.e. patients with evaluable transfusions) regarding demographic characteristics, diagnosis, treatment and transfusion history (table 1). The same applied for the excluded, non-transfused patients in both groups. Splenomegaly was present in 17 patients (10.1%). The mean time on study for patients receiving PAS II PCs and plasma PCs was 20.7 +/- 7.1 and 21.5 +/- 8.6 days (p = 0.54), respectively. Twenty-one patients were randomized more than once (plasma PC n = 11; PAS II PC n = 10). There were no significant differences in the occurrence of febrile episodes, proven infections and mucosal damage, most often localised to the digestive system (painful oral lesions and diarrhoea).

Table 1: *Patient characteristics.*

		Plasma PC (n = 84)	PAS II PC (n = 84)	p-value
	Male / female	53/31	56/28	0.85
	Age (Years ± sd)	51.4 ± 13.1	50.1 ± 14.6	0.54
	Body surface area (m^2 ± sd)	1.94 ± 0.22	1.92 ± 0.24	0.57
	Enlarged spleen	6 (7.1)[1]	11(13.1)	0.31
Diagnosis	AML / MDS	43(51)	44(52)	1.00
	ALL	7(8.3)	5(5.9)	0.77
	CML	5(5.9)	3(3.6)	0.72
	CLL	1(1.2)	3(3.6)	0.62
	Myeloma	14(17)	6(7.1)	0.09
	NHL	13(15)	21(25)	0.18
	Other	1(1.2)	2(2.4)	1.00
Therapy	Remission Induction	31(37)	31(37)	1.13
	Consolidation	9(11)	12(14)	0.64
	Allogenic transplant	18(21)	20(24)	0.58
	Autologous transplant	23(27)	19(23)	0.59
	TBI	17(20)	14(17)	0.69
	ATG	5(5.9)	7(8.3)	0.77
	Other	3(3.6)	2(2.4)	1.00
Transfusion history	RBCs[2]	66(79)	62(74)	0.59
	PCs	58(69)	52(62)	0.42
	Transplants	10(12)	5(5.9)	0.28

[1]*Number of patients (percentage of patients in study arm.* [2]*RBCs = red blood cell concentrates.*

Platelet transfusions: product parameters and increments

A total number of 765 PCs were transfused (plasma PC n = 354; PAS II PC n = 411). A total of 684 PC transfusions could be evaluated (89%; Plasma PC n = 311, PAS II PC n = 373). In the PAS II PC group the 1-hour and 24-hour CCI could be calculated in 337 (90%) and 334 (90%) transfusions, respectively. In the plasma PC group this was the case in 274 (88%) and 282 (91%) transfusions. The missing CCIs were a result of missing data regarding pre count, 1- and/or 24-hour post count.
In table 2 product parameters, dosage, count increments and CCIs are shown.
Although the mean platelet content of PAS II PCs was significantly lower than of plasma PCs, there was no significant difference in the mean dose per kilogram body weight per transfusion between the two groups. There was a significant difference regarding the pH. However, all products had a pH well above 6.8. Univariate analysis, assuming each platelet transfusion as independent event, showed a mean difference in 1-hour and 24-hour CCI between plasma PCs and PAS II PCs off 19.7% (95% CI 11.7 – 27.2%, $p < 0.0001$) and 17.8% (95% CI 5.9 – 31%, $p = 0.004$), respectively. We also analysed the CCIs in a mixed linear model for biological interdependence of consecutive PC transfusions (data shown in table 2). This analysis resulted in the same mean difference in 1-hour and 24-hour CCI between plasma PCs and PAS II PCs, but confidence intervals and p-values differed. In respect to the difference in 1-hour CCI the analysis showed a 95% CI between 6.5 and 32.9% ($p = 0.004$), and the difference in 24-hour CCI resulted in a 95% CI between –2.4 and 38.1% ($p = 0.09$). A multivariate analysis as described in the methods section showed an independent effect of the used storage medium with regard to both count increments and CCIs. Plasma PCs and PAS II PCs resulted in a sufficient 1-hour CCI in 81.3%and 69.1% respectively ($p < 0.0001$). The 24-hour CCI was sufficient in 70.7% and 65.7% ($p = 0.16$). Considering the difference in platelet content of plasma PCs and PAS II PCs, we also performed a linear regression analysis of count increments and platelet dose confirming significant lower 1- and 24-hour count increments after transfusion of PAS II PCs (data not shown). Gamma irradiation had no significant effect on the transfusion responses of both PCs.

Table 2: *Platelet product parameters, dosage and transfusion response.*

		Plasma PC	PAS II PC	p-value
		(n = 311)	(n = 373)	
Numbers of platelets/product	10^9 ± sd	412 ± 93	391 ± 119	p = 0.01
Storage time	days ± sd	3.5 ± 1.3	3.5 ± 1.1	n.s.
pH	± sd	7.12 ± 0.04	7.08 ± 0.04	p < 0.0001
Product volume	ml ± sd	356 ± 19	316 ± 11	p < 0.0001
Precount	10^9/l ± sd	13.3 ± 8.7	13.7 ± 10.5	n.s.
Platelet dose/kg body weight[1]	10^9/l ± sd	5.5 ± 1.7	5.3 ± 2.0	n.s.
Transfusion response[2]				
1-hour		n = 274	n = 337	
CI		32.2 ± 17.1	24.6 ± 14.8	p = 0.001
CCI		13.9 ± 7.0	11.2 ± 6.4	p = 0.004
24-hour		n = 282	n = 334	
CI		20.6 ± 16.0	16.3 ± 14.1	p = 0.028
CCI		8.4 ± 6.9	6.8 ± 6.4	p = 0.09

n = number of transfusions. [1]Per transfusion. [2]General linear mixed model acounting for within-patient-correlation of observations (repeated measurements).

Bleeding complications, transfusion reactions and transfusion interval

The overall incidence of bleeding complications was 32.1%, consisting of 16.1% grade I, 14.3% grade II and 1.8% grade III. Grade IV bleeding was not observed. There were no differences between the two study groups. As a surrogate marker for bleeding we also calculated the mean transfused red cell concentrates per patient, no difference was observed.
A total number of 26 mild transfusion reactions were observed in 21 patients. Of these, 17 (5.5%) transfusion reactions were related to plasma PCs and 9 (2.4%) related to PAS II PCs ($p = 0.04$). Eight patients receiving PAS II PCs experienced transfusion reactions versus thirteen receiving plasma PCs ($p = 0.35$). One patient, receiving plasma PCs, complicated with repeated dyspnoea and wheezing, decided to end the study protocol and was further treated with plasma reduced hyper concentrated platelet products.
Table 3 shows an overview of platelet and red cell transfusions and the calculated transfusion interval. There were no significant differences with regard to transfused PCs, interval and required red cell transfusions per patient. However, the platelet transfusion interval is substantially determined by timing of blood sampling and varying (logistic) delays in PC administration after reaching a transfusion trigger.

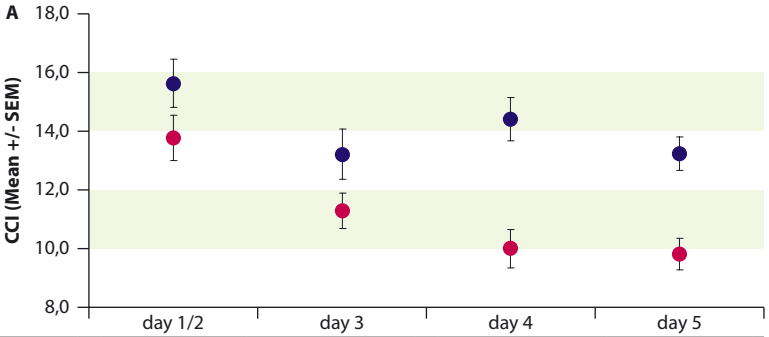
Table 3: *Platelet transfusions, red cell transfusions and transfusion interval.*

	Plasma PC (n = 84)	PAS II PC (n = 84)	p-value
Number of RBC transfusions	452	475	
Mean RBC /patient (± sd)	4.8 ± 4.1	5.1 ± 3.8	0.62
Number of PC transfusions	354	411	
PC transfusion interval (days ± sd)	2.0 ± 1.0	2.1 ± 1.0	0.52
Mean PC/patient (± sd)	4.2 ± 2.7	4.9 ± 2.8	0.10
Cumulative platelet dose/kg (x 10^{11}/kg ± sd)	0.22 ± 0.15	0.23 ± 0.16	0.68

Effects of storage

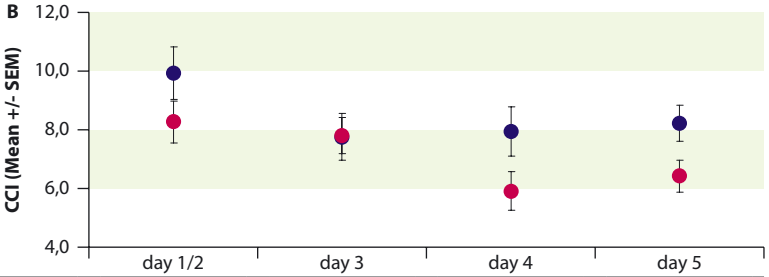
Storage time had a significant effect on the transfusion response of both PCs (figure 1). Stored PAS II PCs as well as stored plasma PCs showed a decrease in 1-hour CCI compared to fresh PCs. For both PCs this difference became significant after two days of storage. Stored plasma PCs show a gradual decrease in 24-hour CCI, however no significance is reached compared to fresh PCs. The same is true for PAS II PCs, although after 4 days of storage the deterioration was significant ($p = 0.02$), showing that the effects of storage time were more pronounced in PAS II PCs.

Figure 1: *Figure 1 A and B shows the comparison of 1- and 24-hour CCI,respectively, related to storage time of plasma PCs and PAS II PCs. Both PCs show a significant decrease in 1- and 24-hour CCI during storage. Figure 2 A showes a significant difference between plasma PCs and PAS II PCs after 3 days of storage. Figure 2 B showes a significant difference beween the two products after 5 days of storage. SEM = Standard Error of the Mean; n = number of transfusions.*



	day 1/2	day 3	day 4	day 5
● Plasma (mean)	15,6	13,2	14,4	13,2
● PAS II (mean)	13,8	11,3	10,0	9,8
p-value	0,11	0,07	0,0001	0,0001
● Plasma (n)	71	66	55	79
● PAS II (n)	83	76	84	87

storage time (days)



	day 1/2	day 3	day 4	day 5
● Plasma (mean)	9,9	7,8	7,9	8,2
● PAS II (mean)	8,3	7,8	5,9	6,4
p-value	0,14	0,96	0,055	0,030
● Plasma (n)	73	66	53	87
● PAS II (n)	82	73	81	87

storage time (days)

Effect factors of increased platelet consumption

Refractoriness, both immunological as well as non-immunological, remains an important clinical problem. In our study 34.5% of patients experienced one or more transfusions with a 1-hour CCI < 7.5, while 25% of all transfusions resulted in a 1-hour CCI < 7.5%. Of all transfusions 25 were major ABO incompatible (plasma PC n = 12, PAS II PCs n = 13), mostly patients with blood group O receiving a blood group A product. A 1-hour transfusion failure after two or more subsequent transfusions occurred in 34 patients (plasma PC n = 14, PAS II PC n = 20), in 7 patients (plasma PC n = 4, PAS II PC n = 3) not explained by obvious non-immunological factors. Testing these patients revealed only one patient with HLA-antibodies and none with HPA-antibodies. Two patients, without detectable HLA-antibodies, received a HLA-matched test transfusion, both without success.

A number of non-immunological factors have been associated with an increase in platelet consumption. Most patients with haematological malignancies experience complex clinical conditions and in our study only 25% of the transfusions were administered in the absence of factors known to increase platelet consumption. A multivariate analysis to evaluate transfusion efficacy in terms of 1- and 24-hour transfusion failure is shown in table 4. Factors independently influencing 1-hour transfusion failure were splenomegaly, ATG, fever and infection. Storage time showed a trend towards an effect, but the used storage medium did not significantly influence the occurrence of 1-hour transfusion failure. The 24-hour transfusion failure was determined by splenomegaly, ATG, fever and the age of the patient significantly contributed to the occurrence of 24-hour transfusion failure, whereas both storage time and used medium dissappeared as independent factors.

Table 4: *Multivariate analysis[1] of 1- and 24-hour transfusion failure.*

	Odds ratio 1-hour CCI < 7.5		Odds ratio 24-hour CCI < 4.5	
	(95% CI)	p	(95% CI)	p
Storage time	1.93 (0.95 – 3.93)	0.069	1.51 (0.82 – 2.79)	0.18
Storage medium	0.60 (0.25 – 1.42)	0.25	0.72 (0.30 – 1.69	0.46
Fever	1.41 (0.97 – 2.04)	0.071	1.88(1.33 – 2.66)	< 0.001
Infection	0.38 (0.17 – 0.84)	0.02	1.08 (0.57 – 2.05)	1.08
Enlarged spleen	26.7 (8.13 – 87.7)	< 0.001	7.55(2.35 – 24.2)	< 0.001
ATG	39.6 (7.81 – 201)	< 0.001	4.83 (1.14 – 20.5)	0.03
Age	*1.01 (0.98 – 1.04)*	0.47	1.04 (1.01 – 1.07)	0.023
Gender	0.59 (0.18 – 1.93)	0.39	0.71 (0.21 – 2.42)	0.58
Diagnosis	0.71 (0.71 – 1.31)	0.96	1.09 (0.82 – 1.43)	0.56
Therapy	1.16 (0.85 – 1.58)	0.36	1.06 (0.79 – 1.44)	0.68
Transfusion history	1.10 (0.41 – 2.90)	0.85	0.68 (0.46 – 3.35)	0.68
Bodyweight	0.97 (0.86 – 1.11)	0.69	0.90 (0.79 – 1.04)	1.04

[1] *Random effects binary logistic model for distinguisable data (odds ratios and p-values are corrected for within-patient-correlation of observations).*

DISCUSSION

With the intention to harmonise platelet products in the Netherlands and in anticipation to future product changes, we performed a randomised controlled trial comparing plasma PCs and PAS II PCs. With the exception of immunological refractoriness due to HLA- and HPA-antibodies no exclusion criteria regarding factors of increased platelet consumption were used. There is general agreement that changes in platelet products should be validated for their clinical quality. Because major bleeding complications are rare, platelet count increments and CCIs have been accepted as surrogate endpoints.[24] A draft guidance for testing and evaluating platelet components advises an array of in vitro tests, the use of in-vivo autologous radio labelled platelet survival studies and clinical trials, including haemostatic efficacy.[25] Currently, in Europe the requirements defined for quality control of platelet transfusion are minimal. In our study swirl, pH and platelet content were determined as in vitro parameters. Swirl was present in all transfused products. The platelet content of the products was measured directly after production as a previous study has shown a limited decline in platelet number during 5 days of storage.[4, 23] We found significant differences with regard to pH and platelet content of the two PCs. The lower pH of PAS II PCs is due to a lower intrinsic pH of PAS II, lower buffering capacity and higher lactate production.[23, 26] The lower platelet content of PAS II PCs can be explained by a viscosity-related difference in the platelet distribution during centrifugation, resulting in a less efficient separation.[23]

We showed that the 1- and 24-hour CCIs of PAS II PCs were lower as compared to plasma PCs, with a mean difference of 19.7% and 17.8%, respectively. This effect remained after correcting for possible confounders in a multivariate analysis. Although the platelet content in PAS II PCs was significantly (approximately 5%) lower as compared to plasma PCs, this small difference is not clinically relevant and the transfused dose per kilogram (or per square meter) in both groups was similar. Univariate analysis of the effect of storage time showed a significant decrease in 1-hour and 24-hour CCI in both products, more pronounced in stored PAS II PCs, in contrast to the results of the study of de Wildt-Eggen.[12] The mechanism of this storage effect is unknown. Increased P-selectin expression and structural changes have been suggested as possible mechanisms.[5, 6] Whether such in vitro changes explain for the inferior increments of PAS II PCs remains unclear.[8, 9]

To investigate the clinical relevance of the inferior CCI of PAS II PCs we compared the incidence of bleeding, transfusion interval, red cell concentrate usage and the occurrence of transfusion failure, the latter also in relation to patient factors. We did not observe significant differences with regard to bleeding complications or the consumption of PCs and red cell concentrates. Univariate analysis of transfusion failures showed a significant effect of PAS II PCs on the occurrence of 1-hour transfusion failure, but not on the 24-hour transfusion failure. A multivariate analysis showed that patient related factors overruled product defined factors as determinants of transfusion failure at 1- and 24-hour, with the exception of storage time, which showed a trend towards 1-hour transfusion failure. The only other randomised study conducted by de Wildt-Eggen12 used a different transfusion threshold (> 20 x 10^9/l) and excluded sick patients. It is likely that the differences in CCIs and transfusion failure between the two studies are caused by factors of increased platelet consumption in our study population, as several studies demonstrated the impact of patient factors on the occurrence of transfusion failure.[15-19] In our study over 75% of all PCs were transfused during episodes with clinical complications associated with increased platelet consumption and multivariate analysis showed that patient related factors annihilated the effects of the used storage medium in relation to transfusion failure.

Compared to other studies we found a relatively low percentage of transfusion reactions, although significantly less after transfusions with PAS II PCs ($p = 0.04$), confirming the results of de Wildt-Eggen.[12] Probably this percentage underestimates the real frequency due to the fact that most reactions are mild, whereas fever and chills are common symptoms in this category of patients.

In conclusion we showed that transfusion responses with PAS II PCs are inferior as compared to plasma PCs. The biological significance of this observation is not significantly exceeding a 30% deterioration. A multivariate analysis showed that patient related factors annihilated the observed differences and there were no significant differences with regard to bleeding complications or PC consumption. Transfusion reactions were mild, infrequent and significantly lower with PAS II PCs. As most patients in need of supportive care temporarily experience factors leading to increased platelet consumption, we propose that future clinical trials studying experimental platelet products should include these patients. We showed safety and efficacy of PAS II PCs in intensively treated patients, however plasma PCs show superior increments. To prevent a downward creep in future developed platelet products, we advise storage of platelets in plasma should be included as a reference in future trials.

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REFERENCES

1. Schiffer CA, Anderson KC, Bennett CL, et al, for the American Society of Clinical Oncology. Platelet transfusion for patients with cancer: Clinical Practice Guidelines of the American Society of Clinical Oncology. J Clin Oncol. 2001; 19: 1519-1538.
2. Slichter SJ. Platelet transfusion: future directions. Vox Sang. 2004; 87 (suppl.2): S47-51
3. de Wildt-Eggen J, Gulliksson H. In vivo and in vitro comparison of platelets stored in either synthetic media or plasma. Vox Sang 2003; 84: 256-264.
4. van der Meer PF, Pietersz RNI, Reesink HW. Storage of platelets in additive solution for up to 12 days with maintenance of good in-vitro quality. Transfusion. 2004; 44: 1204-1211
5. Bunescu A, Hild M, Lundahl J, N. Egberg. Platelet storage in PAS-2 or autologous plasma: impact on functional parameters. Transfus Med. 2001; 11: 105-110.
6. Wagner T, Vetter A, Dimovic N, et al. Ultrastructural changes and activation differences in platelet concentrates stored in plasma and additive solution. Transfusion 2002; 42: 719-727.
7. Evaluation of stored platelets. Vox Sang 2004; 86: 203-223.
8. Cardigan R, Williamson LM. The quality of stored platelets after storage for 7 days. Transfus Med. 2003; 13: 173-187.
9. Dijkstra-Tiekstra M, Pietersz RNI, Huijgens PC. Correlation between the extent of platelet activation in platelet concentrates and in vitro and in vivo parameters. Vox sang. 2004; 87: 257-263.
10. Turner VS, Mitchel SG, Hawker RJ. More on the comparison of Plasm-Lyte A and PAS-2 as platelet additive solutions. Transfusion 1996; 36: 1033.
11. van Rhenen DJ, Vermeij J, Kappers-Klunne M, Payrat JM. Evaluation of a new citrate-acetate-NaCl platelet additive solution for the storage of white cell-reduced platelet concentrates obtained from half-strength CPD pooled buffy coats. Transfusion 1995; 35: 50-53.
12. de Wildt-Eggen J, Nauta S, Schrijver JG, van Marwijk Kooy M, Bins M, van Prooijen HC. Reactions and platelet increments after transfusion of platelet concentrates in plasma or an additive solution: a prospective, randomized study. Transfusion. 2000; 40: 398-403
13. van Rhenen D, Gulliksson H, Cazenave J-P, et al. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood. 2003; 101: 2426-2433.
14. van Rhenen DJ, Gulliksson H, Cazenave J-P, Pamphilon D, Davis K, Flament J. Therapeutic efficacy of pooled buffy-coat platelet components prepared and stored with a platelet additive solution. Transfus Med. 2004; 14: 289-295.
15. Bishop JF, Matthews JP, McGrath K, Yuen K, Wolf MM, Szer J. Factors influencing 20-hour increments after platelet transfusion. Transfusion 1991; 31: 392-396.
16. Norol F, Kuentz M, Cordonnier C, et al. Influence of clinical status on the efficiency of stored platelet transfusion. Br J Haematol 1994; 86, 125-129.
17. Ishida A, Handa M, Wakui M, Okamoto S, Kamakura M, Ikeda Y. Clnical factors influencing posttransfusion platelet increment in patients undergoing hematopoietic progenitor cell transplantation – a prospective analysis. Transfusion 1998; 38: 839- 847.
18. Klumpp TR, Herman JH, Innis S, et al. Factors associated with response to platelet transfusion following hematopoietic stem cell transplantation. Bone Marrow Transplant. 1996; 17: 1035-1041.
19. Slichter SJ, Davis K, Enright H, et al. Factors affecting post-transfusion platelet increments, platelet refractoriness, and platelet transfuison intervals in thrombocytopenic patients. Blood. 2005; 105: 4106-14.
20. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47: 207-214
21. http://ctep.info.nih.gov/reporting/ctc.html
22. Hogmann CF, Eriksson L, Hedlund K, Wallvik J. The bottom and top system: a new technique for blood component preparation and storage. Vox sang 1988; 55: 211-217.

23. van der Meer PF, Pietersz RNI, Tiekstra MJ, Huijgens PC, Dekker WJA, Reesink HW. WBC-reduced platelet concentrates from pooled buffy coats in additive solution: an evaluation of in vitro and in vivo measures. Transfusion 2001; 41: 917-922.
24. Vostal JG, Reid TJ, Mondoro TH. Summary of a workshop on in vivo efficacy of transfused platelet components and platelet substitutes. Transfusion. 2000; 40: 742-750.
25. Guidance for Industry (Draft) for Platelet Testing and Evaluation of Platelet Substitute products. http://www.fda.gov.cber/guidelines.htm.