Chapter 4

Clinical effectiveness of leukoreduced, pooled donor platelet concentrates, stored in plasma or additive solution with and without pathogen reduction

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4

4 Clinical effectiveness of leukoreduced, pooled donor platelet concentrates, stored in plasma or additive solution with and without pathogen reduction

SUMMARY

Pathogen reduction (PR) of platelet products increases costs and available clinical studies are equivocal with respect to clinical and haemostatic effectiveness. We conducted a multicentre, open-label, randomised, non-inferiority trial comparing clinical effectiveness of buffy-coat derived leukoreduced platelet concentrates (PC) stored up to seven days in plasma with platelets stored in platelet additive solution III (PASIII) without and with treatment with amotosalen-HCl/UVA photochemical pathogen reduction (PR-PASIII). Primary endpoint of the study was 1-hour corrected count increment (CCI). Secondary endpoints were 24-hour CCI, bleeding, transfusion requirement of red cells and PC, platelet transfusion interval and adverse transfusion reactions. Compared to plasma-PC, in the intention to treat analysis of 278 evaluable patients the mean difference for the 1-hour CCI of PR-PASIII-PC and PASIII-PC was -31% ($p < 0.0001$) and -9% (p = n.s.), respectively. Twenty-seven patients (32%) had bleeding events in the PR-PASIII arm, as compared to 19 (19%) in the plasma arm and 14 (15%) in the PASIII arm ($p = 0.034$). Despite the potential advantages of pathogen (and leukocyte) inactivation of amotosalen-HCl/UVA-treated platelet products, their clinical efficacy is inferior to platelets stored in plasma, warranting a critical reappraisal of employing this technique for clinical use.

Keywords:

Platelet, Buffy-coat, Amotosalen/UVA Pathogen Reduction, Efficacy.

4 Clinical effectiveness of leukoreduced, pooled donor platelet concentrates, stored in plasma or additive solution with and without pathogen reduction

For the generally accepted indications for treatment and prevention of bleeding, millions of platelet products are transfused yearly, warranting vigilance towards emerging logistical problems and safety issues (Slichter, 2007; Stroncek & Rebulla, 2007). Donor counselling and screening, including molecular techniques, have reduced the risk of transmission of hepatitis B, hepatitis C, HIV, HTLV-I and –II. However, despite the bacterial culture of platelet products, a risk of 1 in 25,000 platelet transfusions for transfusion-related sepsis still remains (Goodnough et al, 1999; Kuehnert *et al*, Dodd *et al*, 2002; Blajchman et al, 2005; Schrezenmeier *et al*, 2007). Availability of platelets and reduction of costs due to reduced outdating would benefit from extending the storage time of platelet products, which is hampered mainly by the risk of bacterial growth beyond 5 days of storage (Lee *et al*, 2003). Pathogen reduction (PR) has been shown to be very effective for the inactivation of several viruses and bacteria (Lin *et al*, 2004; Lin *et al*, 2005). Moreover, PR might also comprise a solution for emerging pathogens, CMV and an alternative for γ-irradiation for the prevention of graft-versus-host-disease (Grass *et al*, 1999; Lin *et al*, 2001). Several countries consider implementing PR as a standard for all platelet products, but concerns still exist with regard to clinical efficacy, potential long-term toxicity as well as uncertainty whether PR- platelet products can be stored longer than 5 days (Council of Europe, 2001; Simonsen *et al*, 2006). Although PR platelet products using amotosalen-HCl and UVA fulfil standard release criteria up to 7 days of storage, this treatment results in considerable metabolic deterioration, increased platelet activation during storage and inconsistent findings by in-vitro haemostatic assessment (van Rhenen *et al*, 2000; Picker *et al*, 2004; Janetzko *et al*, 2004; Jansen *et al*, 2004; Lozano *et al*, 2007; Apelseth *et al*, 2007; van der Meer *et al*, 2009). Nevertheless, transfusion in thrombocytopenic patients corrected prolonged bleeding times (Slichter *et al*, 2006). Radiolabeled, autologous 5 days stored amotosalen-HCl/UVA-treated platelets showed a significant lower recovery and reduction in survival time as compared to platelets stored in PASIII additive solution (Snyder *et al*, 2004). Three randomised controlled trials have been performed using amotosalen-HCl/UVA-treated platelet concentrates (PC) (van Rhenen *et al*, 2003; McCullough *et al*, 2004; Janetzko *et al*, 2005). In the SPRINT trial (645 patients), using aphaeresis PC stored in plasma as control, significantly lower post transfusion platelet increments were found, combined with a reduced transfusion interval and an increased rate of transfusion failure (McCullough *et al*, 2004). The EuroSPRITE trial (103 patients) reported no significant differences with regard to transfusion efficacy, however the control arm of this study used buffy-coat derived platelets stored in plasma as well as in additive solution (PASII) for approximately half of the transfusions (van Rhenen *et al*, 2003). In a previous RCT we have shown that PASII PC have a 20% lower corrected count increment as compared to plasma PC, which might have masked a relevant difference (Kerkhoffs *et al*, 2006). A third small trial with 43 patients showed a borderline significant reduction in transfusion efficacy (Janetzko *et al*, 2005). None of these trials reported inferior haemostatic efficacy. Before the implementation of pathogen reduced platelet products, extending storage time to 7 days while maintaining clinical efficacy is an important aspect to compensate for the additional costs of the procedure. We performed a multicentre open-label, randomised clinical trial to study the clinical efficacy in terms of transfusion response of pooled, random donor PC stored up to seven days in platelet additive solution (Intersol, Fenwal, Inc., Lake Zurich, IL, USA) without additional PR (PASIII) and with amotosalen-HCl/UVA photochemical PR (PR-PASIII, Intercept Blood System, Cerus Corporation, Concord, CA, USA), compared to platelets stored in plasma.

METHODS

Study design

The study was designed as a prospective, randomised open-label non-inferiority trial in haemato-oncological patients with thrombocytopenia or expected to be thrombocytopenic caused by myelosuppression. Patients were included at the haematology wards of eight Dutch hospitals. The study protocol and consent forms were approved both by a central ethics committee as well as local institutional review boards. The study was conducted according to the ICH-GCP guidelines and the declaration of Helsinki. During the study all centres were audited and trial conduct was monitored by an independent organisation. All patients older than 18 years, having a haemato-oncological disease, were eligible for inclusion if they were expected to receive 2 or more platelet transfusions. Exclusion criteria were immunological refractoriness to random platelet transfusions due to HLA- and/or HPA-antibodies or clinical relevant auto-antibodies, pregnancy (or lactating) and previous inclusion in this study. After informed consent eligible patients were registered and randomised, stratified by centre, before start of platelet transfusions in a 1:1:1 ratio to receive per protocol up to a maximum of 5 platelet transfusions with Plasma-PC, PASIII-PC or PR-PASIII-PC in a period of maximal 42 days. Off protocol platelet transfusions were allowed during the study period in case of non-availability of the correct component. Apart from normal completion, reasons to go off study were refusal to continue by the patient or treating physician, intercurrent death and immunological refractoriness.

Platelet products, transfusions and monitoring

All products were produced by the Sanquin Blood Bank. PCs were prepared from 5 pooled whole-blood buffy-coats (BC) with the same ABO-blood group using standard procedures and with regard to pathogen reduction using manufacturer's instructions (van Rhenen *et al*, 2003; Kerkhoffs *et al*, 2006). Samples were obtained prior to storage to measure platelet content. Samples of all products were cultured for 7 days using the BacT/Alert culturing system (BioMerieux, Boxtel, the Netherlands). All products were stored with gentle agitation at 20–24˚C up to seven days. The PCs were γ-irradiated if requested by the hospital.
Indications for platelet transfusions were divided into platelet count-based prophylaxis, intervention related prophylaxis and treatment of bleeding. Generally accepted guidelines were used as guidance for the indication of platelet transfusions. If or when a transfusion was ordered was determined by the treating physician. In summary, in stable, non-bleeding patients a platelet transfusion was advised to maintain the platelet count $\geq 10 \times 10^9$/l and $\geq 40 \times 10^9$/l when these patients receive anti-coagulant therapy or treatment with anti-thymocyte globulin. A transfusion trigger of 40×10^9/l was recommended in endoscopic evaluation of the gastrointestinal or respiratory tract, when no biopsies are performed, diagnostic pleural or peritoneal puncture with a thin needle, lumbar puncture, extraction of a central venous catheter and minor surgical interventions. A trigger of 60×10^9/l was recommended in case of bleeding, endoscopic evaluation with biopsies, dental extractions, placement of a central venous catheter and major surgical interventions, with the exception of neurosurgery and cardiac surgery. In case of cerebral bleeding, diffuse alveolar haemorrhage, neurosurgery and cardiac surgery a trigger of 100×10^9/l was recommended. A pretransfusion platelet count was preferably measured just before transfusion up till a maximum of 6 hours before

transfusion. A 1-hour posttransfusion platelet count was measured between 10 and 120 minutes after transfusion and a 24-hour post transfusion platelet count was measured between 16 and 28 hours after transfusion. The CCI was calculated as follows: $CCI_{1/24\,h}$ = [(post transfusion platelet $count_{1/24\,h}$ – pre transfusion platelet count (x 10^9/L)) x body surface area (m^2)] / platelet dose x 10^{11}. Transfusions given shortly after one another without platelet counts between the transfusions are referred to as multi-dose transfusions and analysed as a single transfusion. If available, ABO-identical PC were used, although minor- and major incompatible PC were not excluded. Platelet transfusion failure was defined as a 1-hour corrected count increment (CCI) below 7.5 and/or and 24-hour CCI below 4.5 (Kerkhoffs *et al*, 2006). Immunological refractoriness was defined as the occurrence of transfusion failure of two consecutive ABO-matched random platelet transfusions combined with existence of HLA- and/or HPA-alloantibodies.

Study endpoints

The primary endpoint was the 1-hour CCI. Secondary endpoints were 24-hour CCI, bleeding, the transfusion requirement of red cells and PCs, platelet transfusion interval and adverse transfusion reactions. The following characteristics were recorded at entry: gender, age, blood group, haematological disease and treatment phase, WHO performance status, existence of enlarged spleen, transfusion history, treatment with anti-coagulation, medical history, medication, bleeding and presence of active infection. The following characteristics were recorded at each transfusion: the reason of the transfusion (trigger, bleeding or intervention), the blood group of the PC, presence of fever, presence of infection (graded according to the CTCAE), presence of mucosal damage, and use of acetaminophen, steroids or antihistamines. Patients were evaluated daily by trained personnel to observe, describe and grade bleeding complications at 8 defined sites according to the CTCAE under supervision of the local investigator (http://ctep.info.nih.gov/reporting/ctc.html). In short grade 1 or minor bleeding comprises petechiae, minimal or microscopic bleeding not requiring interventions. Grade 2 bleeding is defined as gross, symptomatic bleeding for which minimal intervention (i.e. aspiration, cauterisation, irrigation of the urinary tract) is indicated. Grade 3 is severe bleeding requiring red cell transfusions and/or major interventions. Generalized petechiae/purpura as well as retinal bleeding with visual impairment also is classified as grade 3. Catastrophic bleeding defines grade 4, as does CNS bleeding causing neurologic deficit or disability. Lethal bleeding is classified as grade 5. All major bleeding complications were reviewed centrally. Infections were scored in case of positive cultures or if a focus was likely as shown by clinical or radiological examination. Apart from haematological parameters, PT, aPTT and fibrinogen, were measured regularly. Some centres performed routine periodic serological testing of HLA- and/or HPA-alloantibodies, whereas other centres performed these tests only on indication.

Reporting of serious adverse events and Data Safety Monitoring Board

Serious adverse events (SAE) for the purpose of this study were defined as any untoward medical occurrence that resulted in death, a life-threatening event or any other medical condition which might jeopardize the patient or required intervention to prevent more serious sequelae. SAE reporting was mandatory within 24-hours of the initial observation. An independent Data Safety Monitoring Board (DSMB) was installed before the start of the study. An interim analysis was planned after 300 transfusions. All serious adverse events (SAEs) were reviewed by the DSMB. Two criteria for early stopping of an experimental arm were defined: 1. A negative 24-hour CCI (decrement) not caused by immunological factors in more than 20% of the transfusions; 2. Statistically significant more bleeding complications (CTCAE ≥ 2) compared to the Plasma arm.

Power calculation and statistical analysis

The study was designed as a one-sided, non-inferiority study comparing the 1-hour CCI of the transfusions in the PR-PASIII arm and in the PASIII arm with the Plasma arm. Inferiority of an experimental arm was defined as a 20% lower mean 1-hour CCI compared to the Plasma arm. A mean 1-hour CCI of 15.6 and a standard deviation of 6.0 were used based on a previous study (Kerkhoffs *et al*, 2006). For a power of 90% and an alpha of 0.025 (multiple testing) 100 patients per arm were required. In case of multi-dose transfusions, the sum of the platelet content of the PC was used. If one of the PC products differed from the allocated arm, the multi-dose transfusion was considered as not according to protocol. The mean of the storage times of the PC in a multi-dose transfusion was used as the storage time. The 1 and 24 hour counts after the infusion of the last PC of a multi-dose transfusion were used for analysis. To account for the hierarchical structure of the data with a variable number of transfusions per patient, the data were analysed using mixed regression models with random effects for patient and transfusion number. Besides the CCIs, 1 and 24 hour posttransfusion counts were used as endpoints in regression models with as additional covariates besides arm, platelet dose, pretransfusion counts and body surface area of the patient (Davis *et al*, 1999). The data were analysed by intention to treat (ITT) as well as per protocol (PP). To assess safety, the incidence of bleeding complications and adverse reactions were analysed through tabulation. Pearson's chi-square test was used to compare categorical patient characteristics by arm and the Kruskal-Wallis test to compare ordinal or continuous characteristics by arm. A relation between storage time and the post transfusion counts and CCIs was assessed by adding this factor as covariate to the regression models. The association between the patient and transfusion characteristics mentioned above was assessed by adding each of these variables separately as covariate to the regression models. All statistical analyses were performed using Stata.

RESULTS

Patients and platelet transfusions

Inclusion of patients started March 2007. The inclusion of patients in the PR-PASIII group was halted after 92 patients in January 2009 on advice of the DSMB because of lower CCI's ($p<0.0001$) and more bleedings ($p=0.045$) compared to the plasma group. Inclusion of patients in the plasma and PASIII group ended May 2009 and overall 295 patients were randomised. There were 17 non-evaluable patients, resulting in a total of 278 evaluable patients and 1129 transfusion events (fig 1). There were no significant differences in the patient characteristics of the study groups (table 1). 302 transfusion events (27%) were not according to the allocated study arm, more frequent in both study arms. 85% of the off protocol PC were platelets stored in PASII, 15% were platelets stored in plasma. The study products had a lower platelet content, with a mean difference of 6% and 11% for PASIII-PC and PR-PASIII-PC as compared to plasma PC, respectively (table 1, $p < 0.001$).

Figure 1: *Figure 1 shows the randomisation scheme together with evaluable patients, transfusions and endpoints. n = number of patients, n PC = number of single platelet concentrates, n TRF = number of PC transfusion events (includes pooled transfusions). Of the 17 non-evaluable patients 4 patients were non-eligible due to anti-HLA antibodies and 13 patients did not receive any platelet transfusions, without differences between study groups.*

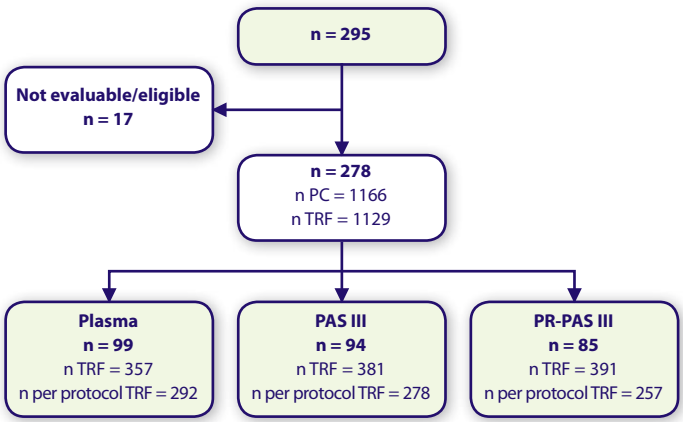

Table 1: *Platelet transfusions, red cell transfusions and transfusion interval.*

	Plasma	PAS III	PR-PAS III
No. of patients	99	94	85
Male / Female	52 / 47	53 / 41	47 / 38
Age, years ± SD	54 ± 12	55 ± 12	53 ± 12
Body surface area, m^2 ± SD	1.93 ± 0.22	1.94 ± 0.19	1.96 ± 0.25
Enlarged spleen N (%)[1]	10 (10)	5 (5)	6 (7)
Diagnosis N (%)			
AML / MDS	42 (42)	52 (55)	44 (52)
ALL	9 (9)	4 (4)	3 (4)
Lymphoma	22 (22)	14 (15)	18 (21)
Multiple myeloma	22 (22)	21 (22)	17 (20)
Other	4 (4)	3 (3)	3 (4)
Therapy N (%)			
Remission induction	47 (47)	46 (49)	39 (46)
Consolidation	5 (5)	6 (6)	3 (4)
Autologous transplantation	32 (32)	31 (33)	33 (39)
Allogeneic transplantation	12 (12)	5 (5)	6 (7)
Other	3 (3)	6 (6)	4 (5)
Transfusion history N (%)			
RBC concentrates	55 (56)	59 (63)	43 (51)
PCs	48 (48)	61 (65)	41 (48)
No. of PC transfusion events	**357**	**381**	**391**
Product type according to protocol (%)	292 (82)	278 (73)[2]	257 (66)[2]
Multi-dose transfusion (%)	14 (4)	12 (3)	11 (3)
PC transfusion indication N (%)			
Prophylactic, trigger based	304 (85)	334 (88)	327 (84)
Intervention	38 (11)	25 (7)	44 (11)
Treatment of bleeding complication	11 (3)	19 (5)	16 (4)
Unknown	4 (1)	3 (1)	4 (1)
Platelet product content, mean x 10^{11} ± SD	3.9 ± 1.0	3.6 ± 0.8[2]	3.4 ± 0.8[2]
Storage time, mean days ± SD	4.0 ± 1.8	3.8 ± 1.8	4.0 ± 1.6
Pre transfusion PLT count x 10^9/L ± SD	18 ± 13	17 ± 13	16 ± 11[3]

[1]Number (%) of evaluable patients and transfusions; [2]p < 0.001 as compared to plasma; [3]p = 0.04 as compared to plasma; AML = Acute myeloid leukaemia; MDS = Myelodysplastic syndrome; ALL = Acute lymphoblastic leukaemia; RBC = Red blood cell; PC = Platelet concentrate. Major ABO-incompatibility occurred in only 6 PC transfusions.

Platelet transfusion efficacy

All efficacy analyses were done ITT as well as PP. The 1-hour CCI and 24-hour CCI were evaluable in 1004 (88.9%) and 1013 (89.7%) of the transfusion events, respectively. The single reason for a non-evaluable CCI-1/24 was failure to perform a platelet count after transfusion and with respect to these missing evaluations there were no significant differences between the study groups or between the per- and off-protocol transfusion events. All transfusion efficacy parameters show inferiority of transfusions with PR-PASIII-PC. There were no significant differences in transfusion responses between PASIII-PC and Plasma-PC (table 2). The proportion of 6 and 7 days stored PC was equally distributed across the arms, being 24%, 21% and 26% of transfused PC in the plasma arm, the PASIII arm and the PRPASIII arm, respectively.

Table 2: *Transfusion response parameters: ITT and according to protocol (PP).*

	Plasma	PAS III	PR-PAS III
No. of patients	99	94	85
ITT analysis			
CCI-1 hour, mean ± SD	17.1 ± 7.3	15.3 ± 6.5	11.4 ± 5.3[5]
Mean diff (97.5% CI)[1]		-9% (-22%; 4%)	-31% (-43%; -18%)
CCI-24 hour, mean ± SD	12.8 ± 7.8	11.6 ± 7.6	7.9 ± 5.3[5]
Mean diff (97.5% CI)[1]		-7% (-26%; 12%)	-34% (-52%; -17%)
PP analysis			
CCI-1 hour, mean ± SD	17.1 ± 7.3	15.3 ± 6.7	10.6 ± 5.0[5]
Mean diff (97.5% CI)[1]		-10% (-23%; 4%)	-36% (-49%; -24%)
CCI-24 hour, mean ± SD	12.5 ± 7.7	11.7 ± 7.6	6.8 ± 5.95
Mean diff (97.5%CI)[1]		-4% (-24%; 16%)	-42% (-61%; -23%)
Other response parameters (ITT)			
CI-1 hour, mean x 10^9/L ± SD	34 ± 15	29 ± 13	20 ± 10[4]
CI-24 hour, mean x 10^9/L ± SD	25 ± 15	21 ± 13	14 ± 10[3]
PC transfusions / patient, mean ± SD	4 ± 2	4 ± 3	5 ± 3[2]
TRF interval (hours), mean ± SD	81 ± 47	77 ± 44	61 ± 47[3]
Transfusion failure (ITT)			
N of Evaluable CCI-1	**314**	**340**	**350**
CCI-1 hour < 7.5 (%)[6]	48 (15)	66 (19)	97 (28)[5]
N of Evaluable CCI-24	**319**	**343**	**351**
CCI-24 hour < 4.5 (%)[6]	72 (23)	94 (27)	125 (36)[3]

The mean CCI and CI values were calculated as the mean of the average CCI/CI of all transfusions per patient.

[1]Mean difference with 97.5% confidence interval of PAS III and PR-PAS III compared to Plasma derived from mixed model regression analyses. [2]p < 0.05, [3]p < 0.01, [4]p < 0.001, [5]p < 0.0001 as compared to plasma; [6]percentage of evaluable CCIs.

Table 3: *Linear regression analysis 1- and 24-hour PLT counts.*

	1-hour PLT count		24-hour PLT count	
	Beta[1]	p-value	Beta	p-value
PASIII	-2.29	0.377	1.79	0.507
PR-PASIII	-9.63	0.001	-8.95	0.003
Storage time (days)	-1.55	<0.001	-1.24	<0.001
Body surface area (m^2)	-15.4	<0.001	-10.1	0.002
Transfusion sequence number	-0.38	0.047	-0.08	0.686
Platelet product content (x 10^9)	0.09	<0.001	0.06	<0.001
Precount (x 10^9/l)	0.96	<0.001	0.96	<0.001

[1]Random effects binary logistic model for distinguisable data (odds ratios and p-values are corrected for within-patient-correlation of 1Beta: regression coefficient. Multivariate linear regression analyses with patient as random factor and as dependent variables the 1-hour platelet (columns 2 and 3) and the 24-hour platelet count (columns 4 and 5). The factors included in the models are shown in the first column. The estimated regression coefficients are shown in the Beta -columns. The regression coefficients measure the strength of the effect per unit change of the corresponding factor; e.g the 1-hour platelet count decreases on average with 1.55 x 10^9/l with each additional day of storage, while an increase of the content of the platelet product with 1 x 10^9 results on average in an increase of 0.09 x 10^9/l of the 1-hour platelet count. The regression coefficients for PAS III and PR-PAS III indicate the average difference in the post transfusion counts as compared to Plasma.).

Both the 1-hour CCI as well as the 24-hour CCI decreased with longer storage time in all study groups. However both CCIs were significantly less in PR-PASIII-PC at each day of storage as compared to plasma PC (figs 2A&B). The 1- and 24-hour CCIs of PASIII-PC did not differ significantly to plasma PC up to 7 days of storage. Linear regression analysis of 1- and 24-hour platelet count showed a platelet dose independent effect of pathogen reduction (figs 2C&D, table 3). A number of product and patient related covariates were tested for an association with CCIs adjusted for arm (table 4). Storage time, enlarged spleen and fever were highly significantly associated with lower CCIs, while the use of steroids as premedication was associated with a higher 1-hour CCI and transfusion for a bleeding indication was associated with a lower 24-hour CCI.

Figure 2: *Figure 2 shows the fitted lines from linear regression analyses restricted to per protocol transfusions. Black, blue and red, respectively represent Plasma, PAS III and PR-PAS III. (2A/B) The 1-hr and 24-hr CCI as function of storage time for the three treatment groups. Point estimates with 95% confidence intervals and number of transfusions are indicated. The lines are the fitted lines assuming a linear relation between CCI and storage time for each group. (2C/D) Fitted 1-hr and 24-hr increments as linear functions of storage time for the three treatment groups for a patient with surface area 1.93, precount 12 and storage time of 4 days. Standard error bars are indicated. (See also supplementary table 1).*

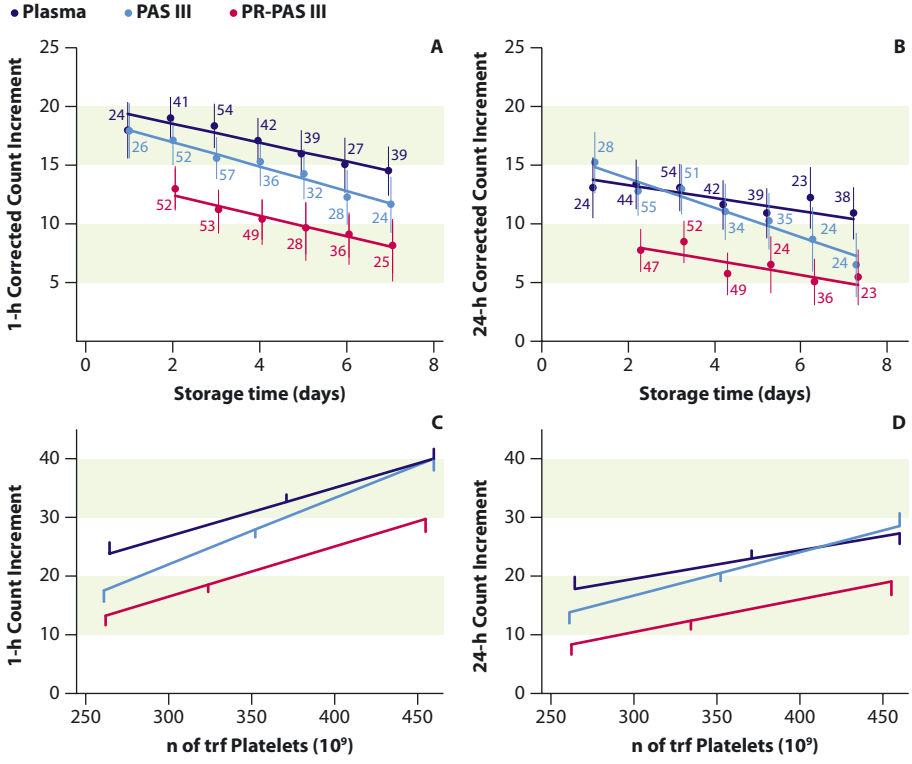

Table 4: *Relation between covariates and the CCI-1 and CCI-24 adjusted for arm.*

	1-hour CCI		24-hour CCI	
	Beta[1] (SE)	p-value	Beta (SE)	p-value
Storage time (days)	-0.9 (0.1)	<0.00001	-0.9 (0.1)	<0.00001
Spleen enlargement	-5.7 (1.4)	<0.00001	-6.5 (1.5)	<0.00001
Fever	-1.7 (0.4)	<0.00001	-1.5 (0.4)	0.0003
Steroids	2.6 (1.1)	0.02	1.0 (1.3)	0.43
Indication bleeding	1.1 (1.0)	0.29	-2.5 (1.1)	0.02
Indication Intervention	-0.6 (0.8)	0.39	-0.4 (0.8)	0.64
Age (years)	0.2 (0.3)	0.49	0.0 (0.3)	0.97
Sex	1.1 (0.8)	0.17	0.3 (0.8)	0.76
Prior PLT TRF	-1.0 (0.8)	0.22	-1.0 (0.8)	0.24
Prior RBC TRF	-0.9 (0.8)	0.25	-0.7 (0.8)	0.42
Prior TRF reactions	-2.4 (1.5)	0.12	-0.3 (1.7)	0.84
Infection	-0.5 (0.5)	0.33	-0.5 (0.5)	0.27
Mucosal damage	-0.1 (0.5)	0.82	0.1 (0.5)	0.82
ABO mismatch	0.2 (0.4)	0.68	0.4 (0.4)	0.33
Anti-histamines	-1.6 (1.3)	0.21	-1.8 (1.3)	0.16
Anti-coagulation	-1.3 (1.3)	0.31	-2.1 (1.4)	0.14
Acetaminophen	1.1 (1.3)	0.39	-1.3 (1.3)	0.31

Univariate random effects regression analysis adjusted for arm. [1]Beta: regression coefficient; SE = Standard error; TRF = Platelet transfusion; RBC = Red blood cell concentrate. All covariates, with the exception of storage time and patient age, are no/yes covariates.

Bleeding and other clinical complications

Sixty-seven new bleeding episodes (CTCAE grade 1-3) were observed in 60 patients during the on study period from the start of the first transfusion with significantly more (p=0.034) and higher grade (p=0.044) bleeding in the PR-PASIII group (table 5).

Distribution of bleeding sites was not different between the study groups. 14 of the bleeding patients were on anticoagulant therapy at the time of bleeding, without differences between the groups. We did not observe lethal bleeding complications in the on protocol period; however, one patient in the PR-PASIII arm deceased due to intracranial bleeding after going off protocol. We did not find an association between platelet dose, storage time or γ-irradiation and the occurrence of bleeding (all grades). There were no differences between the groups with regard to number of RBC transfusions received. The mean number of RBCs in the plasma group was 4±3 as compared to 5±3 and 4±3 in the PASIII and PR-PASIII group, respectively. Twenty-eight mostly mild transfusion reactions occurred in 25 patients, without significant differences between groups (table 5). Incidences of infections and SAE's were equally distributed among the groups. Three SAE's were possibly related to PC transfusion, one in each group. In the plasma group a patient developed a severe, generalized skin reaction, in the PASIII arm a possible TRALI was reported and in the PR-PASIII arm a patient developed acute glottis oedema treated successfully with antihistamines and steroids.

Table 5: *Bleeding, transfusion reactions, infections and SAE's*

	Plasma	PAS III	PR-PAS III
No. of patients	99	94	85
Bleeding after first PC transfusion			
No of patients (%)	19 (19)	14 (15)	27 (32)[1]
No of episodes	19	16	32
Maximum grade (%)			
Grade 1	12 (12)	10 (11)	16 (19)
Grade 2	6 (6)	4 (4)	6 (7)
Grade 3	1 (1)	-	5 (6)
Patients with transfusion reactions, N (%)	11 (11)	8 (9)	6 (7)
No. of transfusion reactions	13	8	7
Severity of events			
No or minor morbidity	11	7	6
Moderate morbidity	1	-	1
Serious morbidity	1	1	-
Patients with infectious complications, N (%)	40 (40)	39 (41)	42 (49)
Maximum grade (%)			
Grade 1 (%)	1	-	-
Grade 2 (%)	3	5	6
Grade 3 (%)	30	29	28
Grade 4 (%)	6	4	8
Grade 5 (%)	-	1	-
Immunological Refractoriness, N (%)	2 (2)	-	2 (2)
Serious adverse events, N	7	3	5
SAE related to PC transfusion	1	1	1
Death, N	3[2]	1	3

Except for the number of bleeding episodes, the numbers in the table reflect numbers (percentage) of patients. For the grades of bleeding and infections the maximum grade is used in case of more than one bleeding episode or more than one infection. [1]*p = 0.034 as compared to plasma;* [2]*1 patient died in the plasma arm 24 days after the last transfusion (the fifth) without SAE report. The cause of death was reported on the off study form as related to the treatment of the underlying disease, with fever presumably due to sepsis.*

DISCUSSION

In a non-selected population of thrombocytopenic haematology patients we studied the transfusion efficacy of PR-PASIII-PCs and PASIII-PCs in terms of increments, transfusion failures, PC consumption and transfusion interval as well as bleeding occurrence and adverse transfusion reactions, compared to plasma-PC. In accordance with the SPRINT trial but in contrast to the EuroSPRITE trial, we observed inferiority of transfusions with PR-PASIII-PC with regard to all transfusion efficacy-related endpoints (van Rhenen *et al*, 2003; McCullough *et al*, 2004). Moreover more patients in the PR-PASIII-PC arm experienced bleeding complications. As reported previously, both study products contained less platelets due to loss of platelets during the production process (McCullough *et al*, 2004; Kerkhoffs *et al*, 2006; Murphy *et al*, 2006; Pineda *et al*, 2006). As CCI might not adequately correct for dose differences between arms, we performed linear regression analysis of the posttransfusion platelet counts with covariates treatment arm, platelet content and storage time, also showing an independent effect of PR-PASIII PC (Davis *et al*, 1999). Using the linear regression analysis we estimate that a PR-PASIII-PC should on average contain 200 x 10^9 platelets extra (i.e. approximately 3 BCs) to achieve a comparable count increment. The relationship between storage time with both CCIs showed a constant difference at each incremental day of storage, suggesting a decreased viability of a fixed number of platelets and a normal disappearance of surviving platelets after treatment with this PR technique. To the same extend as plasma PC, PASIII PC show a decrease in transfusion efficacy up to seven days of storage and no difference in bleeding complications. Our results with regard to lower increments are in agreement with the SPRINT study. The discordance with the EuroSPRITE as well as with a large phase IV trial may be due to the usage of PC stored in PASII in approximately half of the reference group attenuating the results of the reference groups in these other studies (van Rhenen *et al*, 2003; Osselaer *et al*, 2009).

Patients in the PR-PASIII group experienced more and more grade ≥ 2 bleeding compared with both the other arms. The EuroSPRITE and the other smaller European RCT reported no differences between the study arms with regard to bleeding complications (van Rhenen *et al*, Janetzko *et al*, 2005). However in the extended safety report of the SPRINT trial the frequency of grade 2-4 bleeding appeared significantly higher in the PR-arm, 43% as compared to 34% in the control arm ($p = 0.02$) (Snyder *et al*, 2005). It is unlikely that the difference in bleeding complications is solely explained by a lower platelet dose resulting in lower post transfusion platelet peak levels. Estimating approximately one-third non-viable platelets in PR-PC, the platelet dose is still comparable with the low to medium dose applied in a recently presented platelet dose trial showing that bleeding complications did not differ between low, medium or high dose levels of platelets transfused (Slichter *et al*, 2010). Possibly, damage of platelet mitochondrial nucleic acids by PR may not only result in loss of viability of a proportion of platelets, but may impair haemostatic capacity as well (Keuren *et al*, 2006; Apelseth *et al*, 2007). We did not find significant differences in transfusion reactions as observed in larger trials using PR-PASIII PC (Osselaer *et al*, 2008a; Osselaer *et al*, 2008b).

This study has some shortcomings. The number of off-protocol transfusions in the PR-PASIII arm can be regarded as an important limitation of our study. However, performances of both an ITT as well as a PP analysis lead to similar conclusions.

The open label aspect of our study is not expected to influence platelet counts, the primary endpoint of our study, although we cannot completely exclude bias with regard to evaluation of bleeding.

In conclusion, although there are clear advantages and arguments in favour of pathogen reduction techniques to increase transfusion safety, our results warrant a reappraisal of pathogen reduction techniques prior to routine implementation. The process of PR using amotosalen-HCl/UVA likely leads to decreased platelet viability and perhaps compromises haemostatic function, the primary goal of platelet transfusions in high risk patients. A comprehensive survey on the nature and consequences of amotosalen-HCl/UVA-induced platelet damage is needed to understand how this damage can be compensated for in routine transfusion practise.

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