bjh research paper

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

Jean-Louis H. Kerkhoffs,[1,2] Wim L. J. van Putten,[3] Viera M. J. Novotny,[4] Peter A.W. Te Boekhorst,[5] Martin R. Schipperus,[2] Jaap Jan Zwaginga,[6] Lizzy C. M. van Pampus,[4] Georgine E. de Greef,[5] Marleen Luten,[3] Peter C. Huijgens,[7] Anneke Brand[1,6] and Dick J. van Rhenen[1] on behalf of the Dutch – Belgian HOVON cooperative group

[1]Sanquin Blood Bank, Southwest Region, Rotterdam, [2]Haga Teaching Hospital, The Hague, [3]Erasmus University Medical Centre, HOVON Data Centre, Rotterdam, [4]UMC St Radboud, Nijmegen, [5]Erasmus University Medical Centre, Rotterdam, [6]Leiden University Medical Centre, Leiden, and [7]VU Medical Centre, Amsterdam, The Netherlands

Received 26 January 2010; accepted for publication 6 April 2010

Correspondence: Jean-Louis H. Kerkhoffs, Sanquin Southwest Region, Plesmanlaan 1a, 2333 BZ Leiden, The Netherlands.
E-mail: j.kerkhoffs@sanquin.nl

http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=861 http://www.controlled-trials.com/ISRCTN88278819/HOVON+82

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, randomized, non-inferiority trial comparing the clinical effectiveness of buffy-coat derived leucoreduced platelet concentrates (PC) stored for up to 7 d in plasma with platelets stored in platelet additive solution III (PASIII) without and with treatment with amotosalen-HCl/ultraviolet-A (UVA) photochemical pathogen reduction (PR-PASIII). Primary endpoint of the study was 1-h corrected count increment (CCI). Secondary endpoints were 24-h 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-h CCI of PR-PASIII-PC and PASIII-PC was −31% ($P < 0{\cdot}0001$) 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 ($P = 0{\cdot}034$). Despite the potential advantages of pathogen (and leucocyte) 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.

For the generally accepted indications for treatment and prevention of bleeding, millions of platelet products are transfused annually, 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, human immunodeficiency virus, human T-cell lymphotropic virus (HTLV) type 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*, 2001; Dodd *et al*, 2002; Blajchman *et al*, 2005; Schrezenmeier *et al*, 2007). The 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 d 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, 2005). Moreover, PR might also comprise a solution for emerging pathogens, cytomegalovirus and an alternative for γ-irradiation for the prevention of graft-*versus*-host-disease (Grass *et al*, 1999; Lin, 2001). Several countries have considered implementing PR as a standard for all platelet products, but concerns still exist with regard to clinical efficacy and potential long-term toxicity as well as uncertainty as to whether PR- platelet products can be stored for longer than 5 d (Council of Europe expert committee in blood transfusion study group on pathogen inactivation of labile blood products, 2001; Simonsen *et al*, 2006). Although PR platelet products using amotosalen-HCl and ultraviolet-A (UVA) fulfil standard

 doi:10.1111/j.1365-2141.2010.08227.x

release criteria up to 7 d 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; Janetzko *et al*, 2004; Jansen *et al*, 2004; Picker *et al*, 2004; Apelseth *et al*, 2007; Lozano *et al*, 2007; van der Meer *et al*, 2009). Nevertheless, transfusion in thrombocytopenic patients corrected prolonged bleeding times (Slichter *et al*, 2006). Radiolabeled, autologous amotosalen-HCl/UVA-treated platelets stored for 5 d showed a significant lower recovery and reduction in survival time as compared to platelets stored in platelet additive solution III (PASIII) (Snyder *et al*, 2004). Three randomized 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 S-59 Platelet Recovery in Thrombocytopenia (SPRINT) trial (645 patients), that used 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 S-59 Platelet Recovery in Thrombocytopenia in Europe (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 randomized clinical trial (RCT) we showed that PASII PC have a 20% lower corrected count increment (CCI) as compared to plasma PC, which might mask 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 implementing PR platelet products, extension of the storage time to 7 d while maintaining clinical efficacy is an important aspect to compensate for the additional costs of the procedure. We performed a multicentre open-label, randomized clinical trial to study the clinical efficacy, in terms of transfusion response, of pooled, random donor PC stored for up to 7 d in platelet additive solution (Intersol, Fenwal, Inc., Lake Zurich, IL, USA) without additional PR (PASIII) and with amotosalen-HCl/UVA photochemical PR (PR-PAS-III, Intercept Blood System, Cerus Corporation, Concord, CA, USA), compared to platelets stored in plasma.

Methods

Study design

The study was designed as a prospective, randomized open-label non-inferiority trial in haemato-oncological patients with thrombocytopenia or expected to be thrombocytopenic caused by myelosuppression. Patients were recruited from 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 International Conference on Harmonization/World Health Organization (WHO) Good Clinical Practice (ICH-GCP) guidelines and the declaration of Helsinki. During the study all centres were audited and trial conduct was monitored by an independent organization. All adult patients (aged >18 years) with 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 human leucocyte antigen (HLA)- and/or human platelet antigen (HPA)-antibodies or clinical relevant auto-antibodies, pregnancy (or lactating) and previous inclusion in this study. After informed consent eligible patients were registered and randomized, stratified by centre, before platelet transfusions were started in a 1:1:1 ratio to receive per protocol up to a maximum of five platelet transfusions with Plasma-PC, PASIII-PC or PR-PASIII-PC in a period of maximal 42 d. 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 five 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 d using the BacT/Alert culturing system (BioMerieux, Boxtel, the Netherlands). All products were stored with gentle agitation at 20–24°C for up to 7 d. 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 for the indication of platelet transfusions. The requirement for, and timing of platelet transfusion(s) 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 received anti-coagulant therapy or treatment with anti-thymocyte globulin. A transfusion trigger platelet count of 40×10^9/l was recommended in endoscopic evaluation of the gastrointestinal or respiratory tract, when no biopsies were performed, diagnostic pleural or peritoneal puncture with a thin needle, lumbar puncture, extraction of a central venous catheter and minor surgical interventions. A trigger platelet count 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 h before transfusion. A 1-h post-transfusion platelet count was measured between 10 and 120 min after transfusion and a 24-h post-transfusion platelet count was measured between 16 and 28 h after transfusion. The CCI was calculated as follows: $CCI_{1/24\ h}$ = [(post-transfusion platelet $count_{1/24\ h}$ − pre-transfusion platelet count ($\times 10^9$/l)) × body surface area (m^2)]/platelet dose × 10^{11}. Transfusions given shortly after one another without platelet counts between the transfusions were considered to be 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-h CCI below 7·5 and/or and 24-h 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 the existence of HLA- and/or HPA-alloantibodies.

Study endpoints

The primary endpoint was the 1-h CCI. Secondary endpoints were 24-h 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 platelet count, bleeding or intervention), the blood group of the PC, presence of fever, presence of infection (graded according to the Common Toxicity Criteria for Adverse Events, CTCAE, Version 3; http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf), 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 eight defined sites according to the CTCAE under supervision of the local investigator (http://ctep.info.nih.gov/reporting/ctc.html). Briefly, grade 1 or minor bleeding comprised petechiae, minimal or microscopic bleeding not requiring interventions. Grade 2 bleeding was defined as gross, symptomatic bleeding for which minimal intervention (i.e. aspiration, cauterization, irrigation of the urinary tract) was indicated. Grade 3 was severe bleeding requiring red cell transfusions and/or major interventions. Generalized petechiae/purpura as well as retinal bleeding with visual impairment was also classified as grade 3. Catastrophic bleeding defined grade 4, as did central nervous system (CNS) bleeding causing neurological deficit or disability. Lethal bleeding was 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. In addition to haematological parameters, prothrombin time, activated partial thromboplastin time and fibrinogen levels 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 that might jeopardize the patient or required intervention to prevent more serious sequelae. SAE reporting was mandatory within 24 h 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: (i) A negative 24-h CCI (decrement) not caused by immunological factors in more than 20% of the transfusions, (ii) 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-h CCI of the transfusions in the PR-PASIII and PASIII arms with the Plasma arm. Inferiority of an experimental arm was defined as a 20% lower mean 1-h CCI compared to the Plasma arm. A mean 1-h CCI of 15·6 and a standard deviation of 6·0 were used, based on a previous study

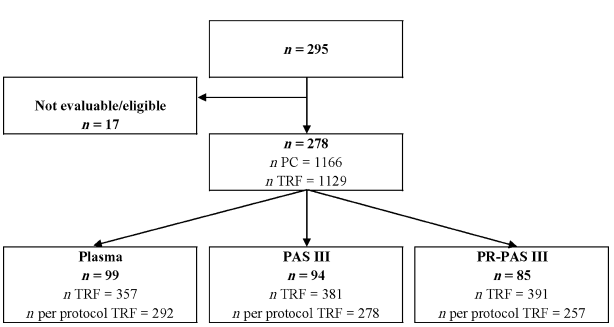

Fig 1. The study randomization scheme, together with evaluable patients, transfusions and endpoints. *n*, number of patients, *n* PC, number of single platelet concentrates, *n* TRF, number of PC transfusion events (includes pooled transfusions). Of the 17 non-evaluable patients, four were non-eligible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

(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-h 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-h post-transfusion 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. The relationship 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

Table I. Characteristics of patients and transfusions.

	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* (%)*	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)†	257 (66)†
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 × 10^{11} ± SD	3·9 ± 1·0	3·6 ± 0·8†	3·4 ± 0·8†
Storage time, mean days ± SD	4·0 ± 1·8	3·8 ± 1·8	4·0 ± 1·6
Pre transfusion platelet count × 10^9/l ± SD	18 ± 13	17 ± 13	16 ± 11‡

Major ABO-incompatibility occurred in only six PC transfusions.

AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukaemia; RBC, red blood cell; PC, platelet concentrate; SD, standard deviation.

*Number (%) of evaluable patients and transfusions.

†$P < 0·001$ as compared to plasma.

‡$P = 0·04$ as compared to plasma.

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

No. of patients	Plasma 99	PAS III 94	PR-PAS III 85
ITT analysis			
CCI-1 h, mean ± SD	17·1 ± 7·3	15·3 ± 6·5	11·4 ± 5·3¶
Mean diff (97·5% CI)*		−9% (−22%; 4%)	−31% (−43%; −18%)
CCI-24 h, mean ± SD	12·8 ± 7·8	11·6 ± 7·6	7·9 ± 5·3¶
Mean difference (97·5% CI)*		−7% (−26%; 12%)	−34% (−52%; −17%)
PP analysis			
CCI-1 h, mean ± SD	17·1 ± 7·3	15·3 ± 6·7	10·6 ± 5·0¶
Mean diff (97·5% CI)*		−10% (−23%; 4%)	−36% (−49%; −24%)
CCI-24 h, mean ± SD	12·5 ± 7·7	11·7 ± 7·6	6·8 ± 5·9¶
Mean difference (97·5%CI)*		−4% (−24%; 16%)	−42% (−61%; −23%)
Other response parameters (ITT)			
CI-1 h, mean × 10^9/l ± SD	34 ± 15	29 ± 13	20 ± 10§
CI-24 h, mean × 10^9/l ± SD	25 ± 15	21 ± 13	14 ± 10‡
PC transfusions/patient, mean ± SD	4 ± 2	4 ± 3	5 ± 3†
TRF interval (h), mean ± SD	81 ± 47	77 ± 44	61 ± 47‡
Transfusion failure (ITT)			
No. of evaluable CCI-1 h	314	340	350
CCI-1 h < 7·5 (%)**	48 (15)	66 (19)	97 (28) ¶
No. of evaluable CCI-24 h	319	343	351
CCI-24 h < 4·5 (%)**	72 (23)	94 (27)	125 (36)‡

The mean corrected count increment (CCI) and count increment (CI) values were calculated as the mean of the average CCI/CI of all transfusions per patient.

ITT, intention-to-treat; PP, per protocol; TRF, transfusion.

*Mean difference with 97·5% confidence interval of PAS III and PR-PAS III compared to Plasma derived from mixed model regression analyses.

†$P < 0·05$, ‡$P < 0·01$, §$P < 0·001$, ¶$P < 0·0001$ as compared to plasma.

**Percentage of evaluable CCIs.

statistical analyses were performed using STATA. P values <0·05 were considered significant.

Results

Patients and platelet transfusions

Patients were included in the study beginning 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 bleeds ($P = 0·045$) compared to the plasma group. Inclusion of patients in the plasma and PASIII group ended in May 2009 and overall 295 patients were randomized. 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 I). A total of 302 transfusion events (27%) were not according to the allocated study arm, more frequent in both study arms. Eighty-five percentage 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 I, $P < 0·001$).

Platelet transfusion efficacy

All efficacy analyses were done ITT as well as PP. The 1-h CCI and 24-h 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 h 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 PP and off-protocol transfusion events. All transfusion efficacy parameters showed inferiority of PR-PASIII-PC transfusions. There were no significant differences in transfusion responses between PAS-III-PC and Plasma-PC (Table II). The proportion PC stored for 6 and 7 d 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. Both the 1- and 24-h 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 (Fig 2A, B). The 1- and 24-h CCIs of PASIII-PC were not significantly different to those of plasma PC up to 7 d of storage. Linear regression analysis of 1- and 24-h platelet count showed a platelet dose-independent effect of PR (Fig 2C, D, Table III). A number of product- and patient-related covariates were tested for an association with CCIs, adjusted for arm

(Tables IV). 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-h CCI and transfusion for a bleeding indication was associated with a lower 24-h CCI.

Bleeding and other clinical complications

A total of 67 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 ($P = 0{\cdot}034$) and higher grade ($P = 0{\cdot}044$) bleeding in the PR-PASIII group (Tables V). Distribution of bleeding sites was not different between the study groups. Fourteen 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 RBC transfusions 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 (Tables V). 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, one patient developed a severe, generalized skin reaction, a possible case of transfusion-related acute lung injury was reported in the PASIII arm and, in the PR-PASIII arm, one patient developed acute glottis oedema that was treated successfully with antihistamines and steroids.

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

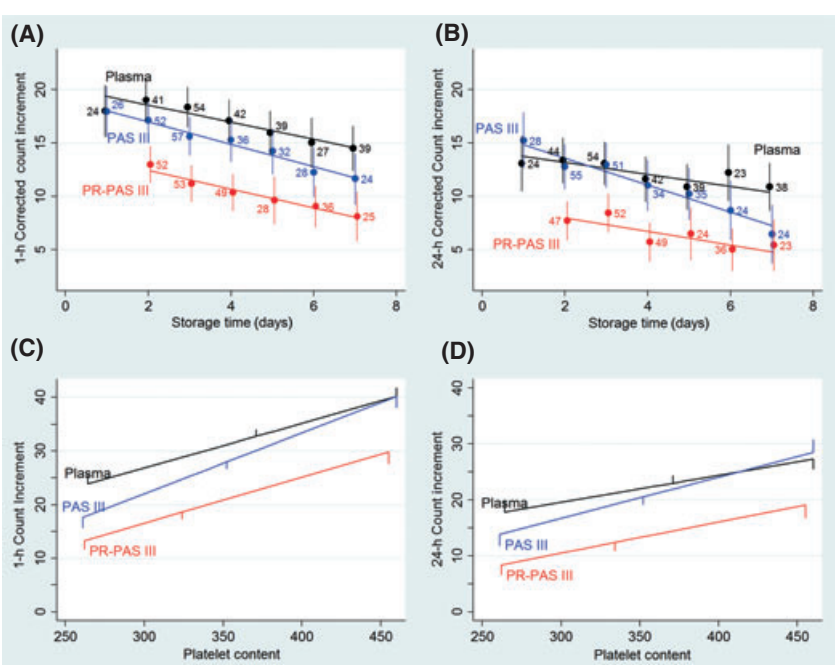

Fig 2. Fitted lines from linear regression analyses, restricted to per protocol transfusions. Black, blue and red represent Plasma, PAS III and PR-PAS III groups, respectively. (A, B) 1- and 24-h CCI as function of storage time for the three treatment groups. Point estimates with 95% confidence intervals and number of transfusions are indicated. The lines are the fitted lines assuming a linear relationship between CCI and storage time for each group. (C, D) Fitted 1- and 24-h increments as linear functions of storage time for the three treatment groups for a patient with body surface area of 1·93 m^2, pre-transfusion platelet count of 12×10^9/l and storage time of 4 d. Standard error bars are indicated.

Table III. Linear regression analysis 1- and 24-h platelet counts.

	1-h platelet count		24-h platelet count	
	Beta*	*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 (d)	−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 ($\times 10^9$)	0·09	<0·001	0·06	<0·001
Precount ($\times 10^9$/l	0·96	<0·001	0·96	<0·001

*Beta: regression coefficient. Multivariate linear regression analyses with patient as random factor and 1-h platelet count (Columns 2 and 3) and the 24-h platelet count (Columns 4 and 5) as dependent variables. The factors included in the models are shown in the Column 1. 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-h platelet count decreases an average of $1{\cdot}55 \times 10^9$/l with each additional day of storage, while an increase of the content of the platelet product with 1×10^9 results in an average increase of $0{\cdot}09 \times 10^9$/l of the 1-h 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.

Table IV. Relation between covariates and the CCI-1 and CCI-24 adjusted for arm.

	1-h CCI		24-h CCI	
	Beta* (SE)	*P*-value	Beta (SE)	*P*-value
Storage time (d)	−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 platelet 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.
All covariates, with the exception of storage time and patient age, are no/yes covariates.
SE, standard error; TRF, transfusion; RBC, red blood cell concentrate.
*Beta: regression coefficient.

not adequately correct for dose differences between arms, linear regression analysis of the post-transfusion platelet counts were performed using the covariates of treatment arm,

Table V. Bleeding, transfusion reactions, infections and serious adverse reactions.

No. of patients	Plasma 99	PAS III 94	PR-PAS III 85
Bleeding after first PC transfusion			
No of patients (%)	19 (19)	14 (15)	27 (32)*
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)
SAEs, *N*	7	3	5
SAE related to PC transfusion	1	1	1
Death, *N*	3†	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.
*$P = 0{\cdot}034$ as compared to plasma.
†One patient died in the plasma arm 24 d after the last transfusion (the fifth) without serious adverse event (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.

platelet content and storage time, which also showed an independent effect of PR-PASIII PC (Davis *et al*, 1999). Using the linear regression analysis we estimated that a PR-PASIII-PC would need to contain an average of 200×10^9 platelets extra (i.e. approximately 3 BCs) to achieve a comparable count increment. The relationship between storage time for both CCIs showed a constant difference at each incremental day of storage, suggesting the decreased viability of a fixed number of platelets and normal disappearance of surviving platelets after treatment with this PR technique. To the same extent as plasma PC, PASIII PC showed a decrease in transfusion efficacy up to 7 d 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 bleeds 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*, 2003; 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 ($P = 0{\cdot}02$) (Snyder *et al*, 2005). It is unlikely that the difference in bleeding complications could be solely explained by a lower platelet dose resulting in lower post transfusion platelet peak levels. Estimating that approximately one-third of platelets were non-viable in PR-PC, the platelet dose was still comparable with the low to medium dose applied in a recently presented platelet dose trial, which showed 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 also impair haemostatic capacity (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, b).

This study has some shortcomings

The number of off-protocol transfusions in the PR-PASIII arm could 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 was 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 PR techniques to increase transfusion safety, our results warrant their reappraisal prior to routine implementation. The process of PR using amotosalen-HCl/UVA probably 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 practice.

Acknowledgements

We would like to thank all the physicians, nurses, technologists, data managers and study coordinators at each study site, as well as all the blood bank personnel who produced all the products. We also thank Cerus Corporation for support in the production of the PR-PASIII PCs.

References

Apelseth, T.O., Bruserud, O., Wentzel-Larsen, T., Bakken, A.M., Bjorsvik, S. & Hervig, T. (2007) *In vitro* evaluation of metabolic changes and residual platelet responsiveness in photochemical treated and gamma-irradiated single-donor platelet concentrates during long-term storage. *Transfusion*, **47,** 653–665.

Blajchman, M.A., Beckers, E.A., Dickmeiss, E., Lin, L., Moore, G. & Muylle, L. (2005) Bacterial detection of platelets: current problems and possible resolutions. *Transfusion Medicine Reviews*, **19,** 259–272.

Council of Europe expert committee in blood transfusion study group on pathogen inactivation of labile blood products. (2001) pathogen inactivation of labile blood products. *Transfusion Medicine*, **11,** 149–175.

Davis, K.B., Slichter, S.J. & Corash, L. (1999) Corrected count increment and percent platelet recovery as measures of posttransfusion platelet response: problems and a solution. *Transfusion*, **39,** 586–592.

Dodd, R.Y., Notari, E.P. & Stramer, S.L. (2002) Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion*, **42,** 975–979.

Goodnough, L.T., Brecher, M.E., Kanter, M.H. & AuBuchon, J.P. (1999) Transfusion medicine: blood transfusion. *New England Journal of Medicine*, **340,** 438–447.

Grass, J.A., Wafa, T., Reames, A., Wages, D., Corash, L., Ferrara, J.L. & Lin, L. (1999) Prevention of Transfusion-Associated Graft-versus-Host Disease by photochemical treatment. *Blood*, **93,** 3140–3147.

Janetzko, K., Lin, L., Eichler, H., Mayaudon, V., Flament, J. & Kluter, H. (2004) Implementation of the INTERCEPT Blood System for platelets into routine blood bank manufacturing procedures: evaluation of aphaeresis platelets. *Vox Sanguinis*, **86,** 239–245.

Janetzko, K., Cazenave, J.P., Klüter, H., Kientz, D., Michel, M., Beris, P., Lioure, B., Hastka, J., Marblie, S., Mayaudon, V., Lin, L., Lin, J.S., Conlan, M.G. & Flament, J. (2005) Therapeutic efficacy and safety of photochemically treated aphaeresis platelets processed with an optimized integrated set. *Transfusion*, **45,** 1443–1452.

Jansen, G.A., van Vliet, H.H., Vermeij, H., Beckers, E.A., Leebeek, F.W., Sonneveld, P. & van Rhenen, D.J. (2004) Functional characteristics of photochemically treated platelets. *Transfusion*, **44,** 313–319.

Kerkhoffs, J.L., Eikenboom, J.C., Schipperus, M.S., van Wordragen-Vlaswinkel, R.J., Brand, R., Harvey, M.S., de Vries, R.R., Barge, R., van Rhenen, D.J. & Brand, A. (2006) A multicenter randomized Study of the efficacy of transfusions with platelet stored in platelet additive solution II versus plasma. *Blood*, **108,** 3210–3215.

Keuren, J.F., Cauwenberghs, S., Heeremans, J., de Kort, W., Heemskerk, J.W. & Curvers, J. (2006) Platelet ADP response deteriorates in synthetic storage media. *Transfusion*, **46,** 204–212.

Kuehnert, M., Roth, V. & Haley, N. (2001) Transfusion-transmitted bacterial infection in the United States, 1998 – 2000. *Transfusion*, **41,** 1493–1499.

Lee, C.K., Ho, P.L., Lee, K.Y., Cheng, W.W., Chan, N.K., Tsoi, W.C. & Lin, C.K. (2003) Estimation of bacterial risk in extending the shelf life of PLT concentrates from 5 to 7 days. *Transfusion*, **43,** 1047–1052.

Lin, L. (2001) Inactivation of cytomegalovirus in platelet concentrates using Helinx technology. *Seminars in haematology*, **38,** 27–33.

Lin, L., Dikeman, R., Molini, B., Lukehart, S.A., Lane, R., Dupuis, K., Metzel, P. & Corash, L. (2004) Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. *Transfusion*, **44,** 1496–1504.

Lin, L., Hanson, C.V., Alter, H.J., Jauvin, V., Bernard, K.A., Murthy, K.K., Metzel, P. & Corash, L. (2005) Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelenght ultraviolet light. *Transfusion*, **45,** 580–590.

Lozano, M., Galan, A., Mazzara, R., Corash, L. & Escolar, G. (2007) Leukoreduced buffy-coat derived platelet concentrates photochemically treated with amotosalen HCl and ultraviolet A light stored up 7 days: assessment of hemostatic function under flow conditions. *Transfusion*, **47,** 666–671.

McCullough, J., Vesole, D.H., Benjamin, R.J., Slichter, S.J., Pineda, A., Snyder, E., Stadtmauer, E.A., Lopez-Plaza, I., Coutre, S., Strauss, R.G., Goodnough, L.T., Fridey, J.L., Raife, T., Cable, R., Murphy, S., Howard, F. IV, Davis, K., Lin, J.S., Metzel, P., Corash, L., Koutsoukos, A., Lin, L., Buchholz, D.H. & Conlan, M.G. (2004) Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. *Blood*, **104,** 1534–1541.

van der Meer, P.F., Kerkhoffs, J.L., Curvers, J., Scharenberg, J., de Korte, D., Brand, A. & de Wildt-Eggen, J.(2009) *In vitro* comparison of platelet storage in plasma and in four platelet additive solutions, and the effect of pathogen reduction: a proposal for an *in vitro* rating system. *Vox Sanguinis*, DOI: 10.1111/j.1423-0410.2009.01283.x.

Murphy, S., Snyder, E., Cable, R., Slichter, S.J., Strauss, R.G., McCullough, J., Lin, J.S., Corash, L. & Conlan, M.G. (2006) Platelet dose consistency and its effect on the number of platelet transfusions for support of thrombocytopenia: an analysis of the SPRINT trial of platelets photochemically treated with amotosalen HCl and ultraviolet A light. *Transfusion*, **46,** 24–33.

Osselaer, J.C., Messe, N., Hervig, T., Bueno, J., Castro, E., Espinosa, A., Accorsi, P., Junge, K., Jacquet, M., Flament, J. & Corash, L. (2008a) A prospective observational cohort safety study of 5106 platelet transfusions with components prepared with photochemical pathogen inactivation treatment. *Transfusion*, **48,** 1061–1071.

Osselaer, J.C., Cazenave, J.P., Lambermont, M., Garraud, O., Hidajat, M., Barbolla, L., Tardivel, R., Defoin, L., Waller, C., Mendel, I., Raidot, J.P., Kandel, G., De Meuter, R., Fabrigli, P., Dehenau, D., Arroyo, J.L., Padrón, F., Gouezec, H., Corral, M., Jacquet, M., Sundin, D., Lin, L. & Corash, L. (2008b) An active haemovigilance programme characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment. *Vox Sanguinis*, **94,** 315–323.

Osselaer, J.C., Doyen, C., Defoin, L., Debry, C., Goffaux, M., Messe, N., Van Hooydonk, M., Bosly, A., Lin, J.S., Lin, L. & Corash, L. (2009) Universal adoption of pathogen inactivation of platelet components: impact on platelet and red blood cell component use. *Transfusion*, **49,** 1412–1422.

Picker, S.M., Speer, R. & Gathof, B.S. (2004) Functional characteristics of buff-coat PLTs photochemically treated with amotosalen-HCl for pathogen inactivation. *Transfusion*, **44,** 320–329.

Pineda, A., McCullough, J., Benjamin, R.J., Cable, R., Strauss, R.G., Burgstaler, E., Porter, S., Lin, L., Metzel, P. & Conlan, M.G. (2006) Pathogen inactivation of platelets with a photochemical treatment with amotosalen HCl and ultraviolet light: process used in the SPRINT trial. *Transfusion*, **46,** 562–571.

van Rhenen, D., Gulliksson, H., Cazenave, J.P., Pamphilon, D., Ljungman, P., Klüter, H., Vermeij, H., Kappers-Klunne, M., de Greef, G., Laforet, M., Lioure, B., Davis, K., Marblie, S., Mayaudon, V., Flament, J., Conlan, M., Lin, L., Metzel, P., Buchholz, D. & Corash, L. (2003) Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euro SPRITE trial. *Blood*, **101,** 2426–2433.

Schrezenmeier, H., Walther-Wenke, G., Müller, T.H., Weinauer, F., Younis, A., Holland-Letz, T., Geis, G., Asmus, J., Bauerfeind, U., Burkhart, J., Deitenbeck, R., Förstemann, E., Gebauer, W., Höchsmann, B., Karakassopoulos, A., Liebscher, U.M., Sänger, W., Schmidt, M., Schunter, F., Sireis, W. & Seifried, E. (2007) Bacterial contamination of platelet concentrates: results of a prospective multicenter study comparing pooled whole blood-derived platelets and apheresis platelets. *Transfusion*, **47,** 644–652.

Simonsen, A.C., Johansson, P.I., Conlan, M.G., Jacquet, M., Lin, J.S., Junge, K., Lin, L., Sørensen, H., Borregaard, N. & Flament, J. (2006) Transfusion of 7-day old amotosalen photochemically treated buffy-coat platelets to patients with thrombocytopenia: a pilot study. *Transfusion*, **46,** 424–433.

Slichter, S.J. (2007) Platelet transfusion therapy. *Hematology/Oncology Clinics of North America*, **21,** 697–729.

Slichter, S.J., Raife, T.J., Davis, K., Rheinschmidt, M., Buchholz, D.H., Corash, L. & Conlan, M.G. (2006) Platelets photochemically treated with amotosalen HCl and ultraviolet A light correct prolonged bleeding times in patients with thrombocytopenia. *Transfusion*, **46,** 731–740.

Slichter, S.J., Kaufman, R.M., Assmann, S.F., McCullough, J., Triulzi, D.J., Strauss, R.G., Gernsheimer, T.B., Ness, P.M., Brecher, M.E., Josephson, C.D., Konkle, B.A., Woodson, R.D., Ortel, T.L., Hillyer, C.D., Skerret, D.L., McCrae, K.R., Sloan, S.R., Uhl, L., George, J.N., Aquino, V.M., Manno, C.S., McFarland, J.G., Hess, J.R., Leissinger, C. & Granger, S. (2010) Dose of Prophylactic Platelet Transfusions and Prevention of Hemorrhage. *New England Journal of Medicine*, **362,** 600–613.

Snyder, E., Raife, T., Lin, L., Cimino, G., Metzel, P., Rheinschmidt, M., Baril, L., Davis, K., Buchholz, D.H., Corash, L. & Conlan, M.G. (2004) Recovery and life span of ^{111}Indium-radiolabeled platelets treated with pathogen inactivation with amotosalen HCl (S-59) and ultraviolet light. *Transfusion*, **44,** 1732–1740.

Snyder, E., McCullough, J., Slichter, S.J., Strauss, R.G., Lopez-Plaza, I., Lin, J.S., Corash, L. & Conlan, M.G. (2005) Clinical safety of platelets photochemical treated with amotosalen HCl and ultraviolet A light for pathogen inactivation: the SPRINT trial. *Transfusion*, **45,** 1864–1875.

Stroncek, D.F. & Rebulla, P. (2007) Platelet transfusions. *Lancet*, **370,** 427–438.

Van Rhenen, D.J., Vermeij, J., Mayaudon, V., Hind, C., Lin, L. & Corash, L. (2000) Functional characteristics of S-59 photochemically treated platelet concentrates derived from buffy coats. *Vox Sanguinis*, **79,** 206–214.