Chapter 5

Clinical efficacy in thrombocytopenic patients of platelets stored in additive solutions as compared to plasma

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Background

The development and introduction of additives for the storage of platelet concentrates (PC) is proceeding steadily. In the Netherlands platelets stored in PAS II (T-Sol) up to 5 days are allowed for transfusion in contrast to platelets stored in plasma, which are allowed to be stored up to 7 days. A recent study suggested an adequate transfusion efficacy with platelets stored in PAS III (Intersol) up to 7 days.

Method

We reanalysed the data of the two RCTs in which plasma PC had been used as control arm and either PAS III PC or PAS II PC as study arms, respectively in order to compare the clinical efficacy of both additive solutions in relation to storage. Moreover, we calculated a combined Odds Ratio for adverse transfusion reactions.

Results

The CCI-1 of PAS II (stored up to 5 days) was 23.6% (95%CI 10.6; 36.5) lower as compared to plasma, whereas PAS III (stored up to 7 days) showed a reduction of 10.9% (95%CI -1.3; 23.2). The same effect was observed with regard to the 24-hour CCIs. Adverse transfusion reactions occurred less frequent after transfusion with platelets stored in an additive solution resulting in a risk reduction of 50% as compared to plasma (95%CI 10 – 72%, $p = 0.025$).

Conclusion

The use of additive solutions reduce the incidence of mild adverse transfusion reactions, an important advantage for patients, and the use of PAS III PCs, stored up to 7 days, for routine transfusion practice is an alternative for PAS II PCs.

Keywords:

platelet concentrates additive solution, efficacy, and adverse reactions

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Since the first publication by Rock et al, the development and clinical use of synthetic additive solutions for the storage of platelets gained interest in many countries with as main incentives the recovery of plasma for other purposes, reduction of adverse transfusion reactions and the improvement of storage conditions to increase the platelet shelf-life.[1, 2] In the Netherlands, the vast majority of platelet products are pooled buffy-coat derived prestorage leukoreduced platelet concentrates (PC), stored either in plasma (Plasma PC) or in PAS II (PAS II PC, Trombosol, Baxter, Lessines, Belgium). Based on a study showing adequate in vitro characteristics and clinical efficacy, storage of plasma PC is allowed up to seven days.[3] Storage of platelets in PAS II is limited to a maximum of five days.[6] In a randomised study, comparing 1 - 5 days versus 6 - 7 stored PAS II PC in transplant recipients a significant decrease in transfusion efficacy of 6 - 7 days stored platelets was shown, without differences in hemorrhagic complications.[9] Although in vitro studies show acceptable quality parameters for PAS II PC during storage up to seven days and the in vivo autologous recovery and survival of 7 days stored PAS II platelets has been reported to be in acceptable ranges this illustrates the limited information of pre-clinical studies.[4] However, it is virtually impossible to compare all different platelet additive solutions in clinical studies. To improve storage conditions other additive solutions have been developed, using differing concentrations of acetate and phosphate, with or without the addition of potassium and magnesium.[10, 11] One of these solutions, PAS III (Intersol, Fenwal Inc., Lake Zurich, Il, USA), differs from PAS II only in the addition of phosphate, which besides increasing buffering capacity, may be superior to PAS II by protection against low adenine nucleoside levels during storage.[12, 13] PAS III PCs as well as PAS II PCs both fulfilled the standard release criteria (pH, swirling) stored up 8 days.[6] We have previously performed two randomised controlled clinical studies, one comparing 1-5 days stored platelets in PAS II with plasma PC and showing an approximately 20% lower efficacy of PASII PC, without a difference in bleeding complications and halving of transfusion reactions. A second RCT, a three-arm study, included Plasma PCs and PAS III PCs both stored up to seven days as control arms.[14] In this study, PAS III PC showed a minor reduction in transfusion efficacy. Instead of conducting a clinical study comparing PAS II with PAS III stored PC for their storage capacity, we analysed the data of these two RCTs in which plasma PC had been used as control arm and either PAS III PC or PAS II PC as study arms.[8, 14]

MATERIALS AND METHODS

Study design

The study design of both trials was very similar with respect to included patients, platelet transfusion policy and study endpoints. The first trial (Trial 1), conducted between October 2003 and April 2005, studied the clinical efficacy of pooled blood, buffy-coat derived platelet products, comparing plasma PCs and PAS II PCs stored up to 5 days.[8] The second trial (Trial 2), conducted between March 2007 and May 2009 compared PAS III PCs treated with pathogen reduction with plasma PCs and PAS III PCs without pathogen reduction.[14] For a detailed description of both trials we refer to the original publications. For both trials all products were produced by the Sanquin Blood Bank, prepared from five pooled buffy-coats with the same ABO-group. Samples of all products were obtained prior to storage to measure platelet count and culture using the BacT/Alert culturing system (Biomerieux, Boxtel, the Netherlands). PCs were stored with gentle agitation at 20 – 24 °C and γ-irradiated at request. There were a number of relevant differences between both trials (table 1). Most importantly, in Trial 1 patients were allowed to be randomised more than once, also more study transfusions were allowed in this trial. The primary objective of this analysis is an indirect comparison of the transfusion efficacy of platelets stored in PAS II (PAS II PC, Trombosol, Baxter, Lessines, Belgium) and platelets stored in PAS III (Intersol, Fenwal Inc., Lake Zurich, Il, USA). For the purpose of this comparison we abstracted the main patient characteristics as well as product characteristics and transfusion efficacy parameters (count increment, corrected count increment) of the first 5 according to protocol transfused PCs from the databases of both studies. We only included the first inclusions in trial 1 (figure 1). Adverse reactions were voluntary reported and classified according to Dutch Hemovigilance guidelines.

Figure 1: *Figure 1 schematically shows the selection of patients and transfusions included for this analysis. PC = Platelet concentrate; TRF = Transfusion; n = number of patients or transfusions.*

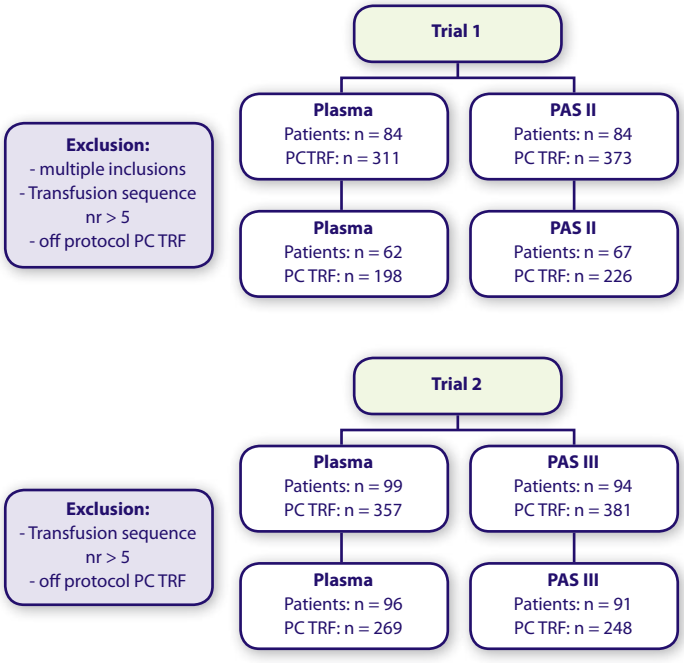

Table 1: *Trial and trial design overview.*

	Trial 1	Trial 2
Period	Oct 2003 – Apr 2005	Mar 2007 – May 2009
Type of trial	RCT, blinded	RCT, non-blinded
Stratification	Yes	Yes
N of study arms	2	3
N of participating centers	2	8
Primary endpoints	CCI-1 and 24-hour	CCI-1 hour
N of evaluable patients	168	278
Type of patients	Hemato-oncology	Hemato-oncology
Age	≥ 18	≥ 18
Exclusion criteria (main)	Auto- and/or alloimmunisation	Auto- and/or alloimmunisation
Multiple inclusions	Yes	No
N of PC transfusions	765	1129
Type of platelet products	BC	BC
Reference product	Plasma	Plasma
Study product	PAS II	PAS III +/- PR
Storage	1 – 5 days	1 – 7 days
N of study transfusions/patient	Maximal 8	Maximal 5

N = number; RCT = Randomised Controlled Trial; PC = Platelet concentrate; BC = Buffy coat; PR = Pathogen Reduction

Statistical analysis

Chi-square tests were used to compare categorical patient characteristics by arm, ordinal and continuous patient were compared using ANOVA. For the statistical comparison of pre- and post transfusion platelet count, count increments (CI) and corrected count increments (CCI) we used an averaged mean per patient to correct for interdependence of consecutive platelet transfusions within a patient. For each of both trials, we performed a multivariate analysis for the effect of several patient variables (sex, age, body surface area, enlarged spleen, pre transfusion platelet count and therapy) and product factors (storage medium, product platelet content and storage time) on post transfusion platelet count. Adverse transfusion reactions were analysed intention-to-treat both on patient as well on transfusion level using the full data set of both trials through tabulation and compared using a chi-square test. All statistical analyses were performed using SSPS (version 15.0 for Windows, Chicago, Il, USA). P-values < 0.05 were considered significant.

RESULTS

PC transfusion characteristics and transfusion efficacy

Randomisation in both RCTs led to well balanced patient characteristics in both studies (table 2). The inclusion of only the PC transfusions of the first inclusion episode of a patient in trial 1 and including only the first 5 on protocol PC transfusions in both trials resulted in 424 PC transfusions to 129 patients in trial 1 and 517 to 187 patients in trial 2. By design PCs in trial 1 had a mean storage time of 3.5 ± 1.2 days as opposed to 4.0 ± 1.9 in trial 2 ($p<0.001$). Comparison of the transfusion efficacy of both plasma PC arms showed several significant differences. As opposed to trial 1, PCs in trial 2 were transfused at a higher pre transfusion platelet count (mean difference 4.2 (1.3 – 6.9)), plasma PCs contained less platelets (mean difference 32 (14 – 51)) and resulted in a significantly higher 24-hour post transfusion platelet count (mean difference 7.3 (2.5 – 12.1)) and 24-hour CCI (mean difference 2.3 (0.3 – 4.4)). For this reason, we decided not to combine the plasma control arms, but to compare both PASs to their respective controls (table 3, figure 2). Both the 1-hour and 24-hour CIs and CCIs of PAS II PCs were significantly lower as compared to plasma. In contrast, only the 1-hour CI of PAS III was significantly lower than plasma. Despite all PCs show a decreased efficacy with increasing storage time, in a multivariate analysis storage interval was a non-significant futile factor in both trials. Only PAS II had an independent negative effect on the 1-hour transfusion efficacy. As is shown in table 4, pre transfusion platelet count and product platelet content are consistently associated with higher post transfusion platelet increment. Body surface area and acute myeloid leukaemia were associated with a decreased post transfusion platelet increment in both trials, whereas an enlarged spleen remarkably only negatively affected the increments in trial 1. Unfortunately we were not informed about the magnitude of the splenomegaly.

Table 2: *Patient and transfusion characteristics*

		Trial 1		Trial 2	
		Plasma	PAS II	Plasma	PAS III
n Patients		**62**	**67**	**96**	**91**
Sex	M/F	39 / 23	45 / 22	50 / 46	52 / 39
Age	Years ± SD	53 ± 14	49 ± 14	54 ± 13	54 ± 13
Body surface area	M^2 ± SD	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2
Acute myeloid leukemia	N (%)	29 (47)	30 (45)	42 (44)	51 (56)
Remission induction Ctx	N (%)	28 (45)	28 (42)	46 (48)	44 (48)
Enlarged spleen	N (%)	5 (8)	8 (12)	10 (11)	5 (6)
n Platelet transfusions		**198**	**226**	**269**	**248**
Storage time	Days ± SD	3.5 ± 1.1	3.5 ± 0.8	4.0 ± 1.5	3.7 ± 1.4
Platelet content	10^9 ± SD	408 ± 62	390 ± 88	376 ± 50	355 ± 43[1]

Ctx = Chemotherapy; [1]p < 0.05 as compared to the respective plasma arm.

Table 3: *Transfusion efficacy.*

	Trial 1			24-hour CCI		
	Plasma	PAS II	p-value[1]	Plasma	PAS III	p-value[1]
Pre count	13 ± 7	14 ± 9	0.425	17 ± 11	15 ± 9	0.174
1-hour						
CI (10^9/l)	33 ± 15	25 ± 12	0.001	34 ± 15	28 ± 13	0.012
CCI	15.7 ± 5.9	12.0 ± 5.5	<0.001	17.0 ± 7.4	15.2 ± 6.6	0.079
24-hour						
CI (10^9/l)	21 ± 11	16 ± 11	0.017	24 ± 14	21 ± 13	0.189
CCI	10.0 ± 5.0	8.0 ± 5.0	0.026	12.3 ± 7.8	11.7 ± 7.7	0.605

[1]*Univariate p-value correcting for interdependence of consecutive PC transfusions using an Averaged mean per patient.*

Figure 2: *Figure 2 shows the estimated mean difference and 95% confidence interval for the 1- and 24-hour CCIs comparing both PASs to their own Plasma control. The dashed lines represent the non-inferiority margins as were used in both Trials.*

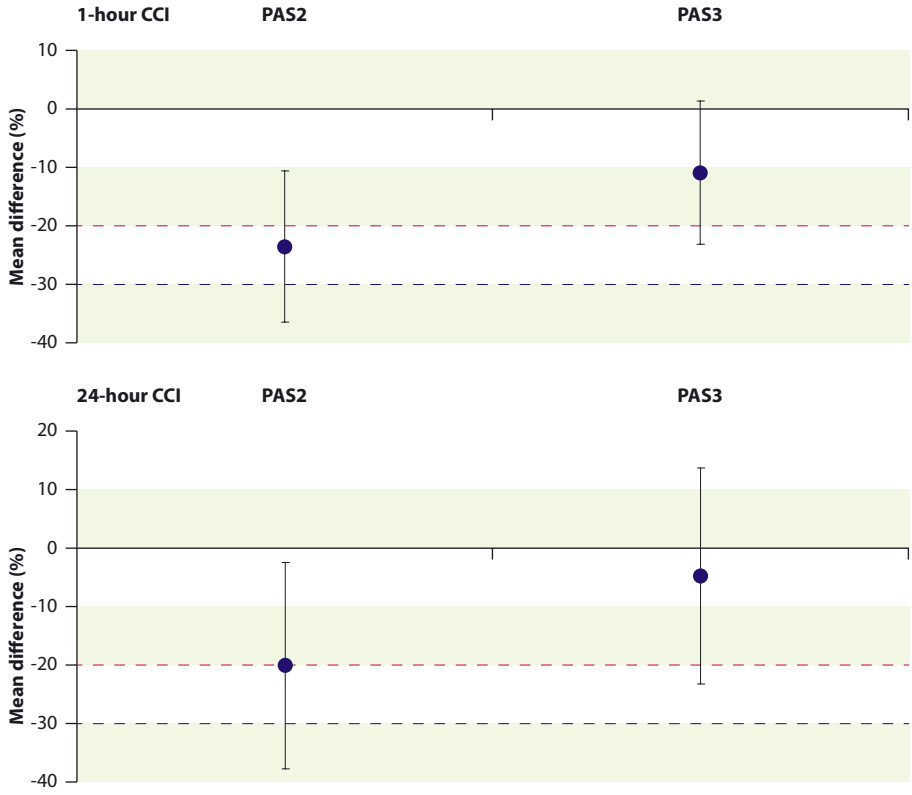

Table 4: *Multivariate analysis of post transfusion PLT count.*

	Trial 1		Trial 2	
Post count	1-hour	24-hour	1-hour	24-hour
Additive solution	-7.07 (-11.1; -3.00)	-3.02 (-6.72; 0.69)	-2.95 (-6.93; 1.02)	-2.31 (-6.36; 1.73)
Pre count	0.72 (0.46; 0.97)	0.90 (0.66; 1.13)	0.80 (0.59; 1.00)	0.90 (0.69; 1.11)
Storage time	-2.19 (-4.50; 0.11)	-0.609 (-2.69; 1.48)	-1.02 (-2.35; 0.31)	-0.49(-1.91; 0.93)
PLT content	0.05 (0.02; 0.08)	0.04 (0.02; 0.07)	0.08 (0.04; 0.12)	0.00 (-0.04; 0.05)
BSA	-20.2 (-28.9; -11.5)	-11.0 (-19.0; -3.06)	-24.2 (-34.1; -14.3)	-12.7 (-22.9; -2.58)
Female	-2.28 (-7.42; 2.87)	0.63 (-4.10; 5.35)	2.76 (-1.86; 7.38)	-0.90 (-5.82; 4.01)
Enlarged spleen	-11.4 (-18.2; -4.51)	-7.37 (-13.7; -1.09)	-1.22 (-3.26; 0.81)	-1.22 (-3.31; 0.87)
AML	-2.21 (-6.31; 1.90)	-4.16 (-7.87; -0.44)	-4.71 (-8.65; -0.77)	-4.37 (-8.47; -0.27)
RI Chemotherapy	3.04 (-2.04; 8.12)	1.65 (-3.00; 6.30)	1.87 (-2.65; 6.39)	-0.65 (-5.33; 4.03)

Beta: regression coefficient. Multivariate linear regression of the 1- and 24 post transfusion PLT count (averaged mean per patient in both trials). The estimated regression coefficient is shown in the columns, measuring the strength of the effect per unit of change of the corresponding factor. BSA = Body Surface Area; AML = Acute Myeloid Leukaemia; RI = Remission Induction.

Adverse transfusion reactions

Transfusion reactions were a secondary endpoint in both RCTs. In both trials the vast majority of adverse transfusion reactions were mild without significant morbidity. An intention-to-treat analysis, combining the results of both trials showed that 9.0% of the patients randomised to receive platelets stored in additive solution experience transfusion reactions, without differences between the type of PAS, as compared to 13.1% of patients randomised to receive plasma stored platelets (OR 0.7, 95%CI 0.3 – 1.3). In the combined additive arms 2.2% of the PC transfusions resulted in an adverse transfusion reaction as compared to 4.5% in the plasma arms (OR 0.5, 95%CI 0.3 – 0.9). Limiting this analysis to the selection of patients and transfusions evaluated in this study the OR for patients treated with additive stored platelets to experience a transfusion reaction is 0.4 (95%CI 0.2 – 1.0) and the OR for additive stored PCs to result in an adverse reaction 0.6 (95%CI 0.3 – 1.1).

DISCUSSION

For blood bank logistical and economical reasons, the use of an additive solution allowing for storage up to 7 days would be very attractive. By analysing the data of two trials, we have compared the transfusion efficacy of PAS II PCs and PAS III PCs relative to their own plasma PC controls. Because comparison of both control arms showed several significant differences we did not choose to pool the plasma controls, which would have enabled a direct comparison. The CCI-1 of PAS II (stored up to 5 days) was 23.6% (95%CI 10.6; 36.5) lower as compared to plasma, whereas PAS III (stored up to 7 days) showed a reduction of 10.9% (95%CI -1.3; 23.2). The same effect was observed with regard to the 24-hour CCIs. As was previously reported there were no haemostatic consequences of the observed decrease in transfusion efficacy, nor did the decreased efficacy lead to differences in transfusion interval or number of PC transfused.[8, 14] Mild adverse transfusion reactions occurred less frequent after transfusion with platelets stored in an additive solution in both trials and combining both trials results in an estimated risk reduction of 50% (95%CI 10 – 72%, p = 0.025).

A trial, comparing PAS II PCs stored 1-5 days with PAS II PCs stored 6-7 days in a paired fashion, showed that the mean 1- and 24-hour CCI of 6-7 day stored PAS II PCs was 7.4 ± 3.8 and 2.6 ± 2.6, respectively.[9] Both these mean CCI values could be conceived as transfusion failures. We did not study 6-7 days stored PAS II platelets, but we estimate by extrapolation of our data (estimated mean CCI-1 and CCI-24 for 6 – 7 day stored platelets in PAS II of 6.1 and 4.9 respectively), consistency with the data from Diedrich et al.[9] The results of our analysis strongly suggest that platelets stored in PAS III have superior clinical efficacy compared to PAS II stored PC and enable extension of storage time to 7 days without a clinically relevant decrease in transfusion efficacy.

The main limitation of this study is the indirect nature of the comparison; despite at first glance both trials appear very similar, there are a number of important differences potentially affecting efficacy such as pre-transfusion platelet count and platelet content of the product and these could not be corrected for by better matching and thus prohibited pooling of the plasma PC arms from the two RCTs.

Nevertheless the results support to replace PASII PC by PAS III PCs, stored up to 7 days, as an acceptable alternative for plasma PCs for routine transfusion practice.
The development of additives with the addition of potassium and magnesium to PAS III are expected to further improve platelet storage conditions.[16] Lacking informative pre-clinical methods however, new platelet products need to be tested for their efficacy as well as haemostatic properties compared to plasma PCs, still gold standard, in clinical studies to avoid as formulated by Scott Murphy a downward creep.

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