Quality assessment and transfusion efficacy of buffy coat-derived platelet concentrates washed with platelet additive solution

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Background. Transfusion of washed platelet concentrates (W-PC) is recommended for some patients, such as those who have had previous severe allergic transfusion reactions. However, we still lack a standardised method for preparing these products. Here, we assessed the effect of a manual washing procedure on *in vitro* platelet quality and on the transfusion efficacy of W-PCs.

Materials and methods. Buffy coat-derived W-PC in Composol solution were prepared by one-step centrifugation. Platelet activation and function were evaluated before and after washing by means of: (i) CD62 expression by flow cytometry; (ii) platelet aggregation (LTA); and (iii) the VerifyNow® P2Y12 test. A pilot prospective transfusion study was carried out in 11 onco-hematology patients receiving, in a short time, two consecutive transfusions: one with standard PC (S-PC) and one with W-PC. The post-transfusion platelet increment, the 1 h and 24 h corrected count increment (CCI) and occurrence of bleeding events were used as indices of transfusion efficacy.

Results. Platelet recovery in W-PC was 84.8±5.4%. Washing slightly increased platelet activation in W-PC *vs* pre-washed samples (% CD62+ platelets 23.6±7 *vs* 14.8±1; p=0.03). As compared to prewash samples, platelet reactivity of W-PC as measured by VerifyNow® P2Y12 was significantly lower with ADP (PRU 32.2±37.7 *vs* 4.2±2.4, p=0.027), but similar using TRAP. Platelet aggregation responses to TRAP, collagen, ristocetin and arachidonic acid were maintained in W-PC. The pilot transfusion trial showed similar 1 h (13.5±5.6 *vs* 11.5±7.3, p=0.49) and 24 h (11±7.2 *vs* 9±6.5, p=0.48) CCI for S-PC and W-PC. Transfusion of W-PC was not associated with an increased number of bleeding events.

Discussion. We have set up a simple method to obtain buffy-coat-derived W-PC, which has minor effects on *in vitro* platelet quality and transfusion effectiveness. This procedure can be easily implemented in transfusion centres for on-demand preparation of washed platelets.

Keywords: washed platelet concentrates, washed platelet function, washed platelet transfusion, VerifyNow® system.

Introduction

Allergic transfusion reactions are among the most frequent non-haemolytic adverse effects of blood component transfusions, with incidence rates varying from about 0.15% for plasma and red cell concentrates up to 5% for platelet transfusions[1,2]. Although the pathophysiology of allergic transfusion reactions remains to be fully elucidated, plasma proteins such as IgA or haptoglobin, food allergens and inflammatory cytokines and chemokines, which accumulate in blood products during storage, have appeared as major mediators of this transfusion complication[1]. Even though allergic transfusion reactions are rarely serious, their prevention is desirable because they would add unnecessary suffering to patients. Often, prophylactic treatment with anti-histamines is used to prevent allergic reactions to platelet transfusions, despite there not being conclusive evidence regarding the preventive value of these drugs[1,3]. On the other hand, plasma reduction and component washing has been shown to be effective in reducing, by as much as 10-fold, the rate of allergic transfusion reactions in patients receiving platelet transfusions[2,4]. Thus, these blood banking techniques might be the new standard of care for all patients or at least for those cases with recurrent allergic transfusion reactions.

Essentially, platelet washing is a process aimed at replacing the donor's plasma in the platelet product

by physiological saline or a platelet additive solution. Unfortunately, this washing process is time-consuming, needs qualified personnel and is hampered by a lack of an international quality standard[5,6].

The aim of our study was to validate a simple protocol for washing standard buffy coat-derived platelet concentrates with platelet additive solution. To do this, we evaluated the effects of washing on *in vitro* activation and functional status of platelets, and compared the transfusion outcome of standard and washed platelets in a cohort of patients who received two platelet transfusions in a short time interval.

Materials and methods

Preparation, washing, and sampling of platelet concentrates

Leucodepleted platelet concentrates (PC) were prepared by pooling five isogroup ABO fresh buffy-coats and 300 mL CompoSol® platelet additive solution (Fresenius Kabi AG, Bad Homburg, Germany), using the OrbiSac system (Terumo BCT Europe N.V, Zaventem, Belgium), essentially as detailed elsewhere[7]. Upon preparation these PC were left without agitation for about 1 h and then stored in a standard blood bank PC incubator (Helmer Inc, Noblesville, IN, USA) set at 22 °C with moderate flat-bed shaking (60 cycles/min) until use for platelet transfusion within 5 days.

The control arm of the study included 11 standard PC (S-PC) which were stored in a PC incubator, set at 22 °C with continuous, gentle agitation and distributed for transfusion to selected patients within 5 days of preparation (mean storage 2.64±1.36 days). On the day of transfusion, these PC were sampled in a sterile manner for *in vitro* evaluation as described below. The investigational arm of the study included another 11 S-PC that were stored equally to those in the control arm (mean storage 2.36±0.66 days) but that, on the day of transfusion, were submitted to a washing process to obtain washed PC (W-PC). Briefly, 300 mL of CompoSol were transferred to the PC using a sterile connecting device (TSCD®-II, Terumo BCT Europe N.V.). The empty CompoSol bag was substituted by a transfer bag (2L Bag BB*J200BM, Terumo BCT Europe N.V.), again using the TSCD®-II, and the PC-transfer sealed bag set was centrifuged at 1,800 *g* for 9 min, at 22 °C in a Sorvall™ RC 3BP centrifuge (Thermo Fisher Scientific Inc. Waltham, MA, USA). The PC supernatant was then carefully transferred into the transfer bag using a manual plasma expressor and a new 300 mL CompoSol bag was connected to the PC with TSCD®-II. The CompoSol solution was added to the PC and the empty bag removed by means of a thermic tube sealer device (T-SEAL II; Terumo BCT Europe). The resultant W-PC was left without agitation for 45 min and then stored for 1 h in the PC incubator with flat-bed shaking (60 cycles/min) to allow platelet resuspension. Finally, the W-PC were distributed for transfusion to selected patients within 4 h of preparation.

These W-PC units were sampled before and after washing for *in vitro* platelet evaluation as described below. In two out of the 11 W-PC, the protein concentration in the PC supernatant was determined before and after washing with an automatic analyser (Cobas 701; Roche España, Barcelona, Spain) and a 90% reduction was found (0.3 *vs* 2.7 mg/dL before washing).

Test and assays

In vitro studies were performed on all PC samples and post W-PC on the day of transfusion to patients. Cell counts in the PC samples were determined by means of an electronic particle counter (STKS, Coulter Electronics, Hialeah, FL, USA).

For the platelet functional studies, 5 mL of PC samples were obtained aseptically and centrifuged (1,000 *g*, 10 min) before removing the platelet-poor supernatant. The platelet pellet was gently re-suspended in the appropriate volume of AB fresh-frozen plasma, in order to adjust the platelet count to 300×10^9 platelets/L. A single batch of AB fresh-frozen plasma was used in the study: the batch was stored at −80 °C and the required aliquot was thawed at 37 °C immediately before use.

Platelet aggregation assays and flow cytometric assessment of platelet activation (P-Selectin [CD62P]), were performed in the platelet suspensions obtained from the PC as described previously[7]. The agonists and concentrations used in aggregations were: 1.6 mM arachidonic acid (AA; DiaMed, Cressier, Switzerland), 25 μM thrombin receptor activating peptide (TRAP; Sigma-Chemical, Madrid, Spain), 1.25 mg/mL ristocetin (Sigma-Chemical) and 2 μg/mL collagen (Menarini Diagnostics, Barcelona, Spain).

Platelet aggregation was also measured using a VerifyNow® system (Accumetrics, San Diego, CA, USA). Briefly, blood was reconstituted *in vitro* using platelets obtained from samples before and after washing, mixed with AB plasma and O red blood cells and were added to $P2Y_{12}$ cartridges to evaluate platelet aggregation as instructed by the manufacturer[8].

In vivo studies

We also performed a prospective transfusion study in our Clinical Unit at the Morales Meseguer University Hospital. The study involved 11 patients with thrombocytopenia induced by chemotherapy or due to haematological diseases, who were expected to receive two platelet transfusions in a short period of time. The order of the platelet transfusion type, first W-PC and second S-PC or *vice versa*, was dependent on the

availability of W-PC in the blood transfusion centre. The primary end-points of the study were post-transfusion platelet increment and 1 h and 24 h corrected count increment (CCI). According to previously described criteria, transfusions were considered to be successful when 1 h and 24 h CCI were at least 7.5×10^9/L and 4.5×10^9/L, respectively[9,10]. Secondary outcome was bleeding events graded according to the World Health Organisation (WHO) classification[11]. Patients' medical records were reviewed and clinical factors that might have influenced transfusion efficacy, including fever, sepsis, mucositis or any recognised clinical toxicity, were evaluated according to WHO criteria[12].

This investigation followed the principles of the Helsinki Declaration and was approved by the hospital Ethics Committee. Patients had provided consent to the use of their medical records for research purposes.

Statistical analysis

The results of the different variables analysed are presented as means ± standard deviations or percentages, as appropriate. The Kolmogorov-Smirnov test was used to check for the normal distribution of data. Differences in the *in vitro* platelet tests between pre- and post-wash PC, and changes in transfusion efficacy between S-PC and W-PC were assessed by paired *t*-tests. Patients' clinical features at the time of the first and second transfusions were compared by Fisher's exact test. p-values <0.05 were accepted as statistically significant. All analyses were carried out using a software programme (SPSS for Windows, Ver. 15; SPSS, Inc., Chicago, IL, USA).

Results

Effect of platelet washing on cell recovery and platelet function

The general characteristics of the PC are summarised in Table I. There were not significant differences in any parameter between S-PC and pre-processing W-PC, suggesting no bias in the selection of standard PC to be washed. As shown, the washing procedure led to a moderate but significant loss of platelets, providing a recovery of approximately 85% of the original platelet content, in a similar product volume.

Under our experimental conditions, washing also induced a mild but significant increase in the platelet activation status, as assessed by P-selectin expression in unstimulated platelets. Moreover, washed platelets displayed a slightly lower capacity to release P-selectin from α-granules upon stimulation with TRAP (Table I).

Light transmission aggregometry, the gold standard for testing platelet function[13], demonstrated that the washing procedure had negligible effects on the functional status of the PC. Indeed, for all tested agonists, washed platelets showed maximal aggregation responses that were similar or even slightly higher than those of their non-washed counterparts. In contrast, in aggregation assays with the point-of-care VerifyNow® test we found that platelet aggregability induced with the weak agonist ADP, but not with iso-TRAP, was significantly impaired after the washing process (Table I).

Effect of platelet washing on transfusion efficacy.

The transfusion efficacy of platelets obtained with our simple washing procedure was assessed in a small cohort of 11 onco-haematology patients (9 men and 2 women), expected to require at least two consecutive transfusions in a short period of time: Two of these patients had received induction chemotherapy for acute myeloid leukaemia, one was under treatment for a secondary acute myeloid leukaemia after an allogeneic transplant, four had received chemotherapy for autologous transplantation and three for allogeneic progenitor cell transplants, and one patient had a myelodysplastic syndrome with severe thrombocytopenia. The median age of the patients was 55.6±8.5 years, and their median body surface was 1.86±0.24 m^2.

Table I - *In vitro* characteristics of standard and washed platelet concentrates.

Parameter	Standard	Washed	
		Pre-manipulation	*Post-manipulation*
Volume (mL)	365±20	366±11	353±20#
Platelets			
($\times10^9$/L)	1,090±100	1,152±161	1,009±113#
($\times10^9$/pool)	397±30	423±63.6	356±41#*
CD62 (% platelet+)			
Basal	18.7±7	14.8±1.8	23.6±7#
TRAP-stimulation	88.7±10.8	88.6±6.8	85.0±7.3#
VerifyNow® P2Y12 (PRU)			
ADP-induced		32.2±37.7	4.2±2.4#
TRAP-induced		275.2±55.9	263.3±63.3
% maximal aggregation			
1.6 mM AA	87.2±4.9	87.3±6.1	83.5±9.3
1.25 mg/mL ristocetin	93.9±6.6^	94.9±6.8^	102.8±6.3
2 μg/mL collagen	87.7±8.7	84.7±8.2	90.3±11.5
25 μM TRAP	89.0± 9.1^	91.0±5.8^	95.2±3.3

All values are mean ± standard deviation from 11 different units. A Student's *t*-test was used to compare standard platelet concentrate (S-PC) values and pre-manipulation washed platelet concentrate (W-PC) values with post-manipulation W-PC values. * p<0.05 *vs* S-PC; # p<0.05 *vs* pre-manipulation W-PC. ^p<0.05 *vs* post-manipulation W-PC. AA: arachidonic acid; ADP: adenosine diphosphate; PRU: P2Y12 reaction units; TRAP: thrombin receptor activating peptide.

In ten out of the 11 patients included, the first platelet transfusion was a W-PC and the second was a S-PC. The mean interval between the first and second transfusions was 3.2±1.9 days. As summarised in Table II, the review of clinical records demonstrated that clinical status of the patients before the first and second transfusions was similar with regards to variables that could influence transfusion efficacy, such as fever, diarrhoea, mucositis, sepsis or active bleeding. We found that 55% of patients entering the study had mucositis the day of transfusion, with no statistical differences in the severity of mucositis before transfusion of S-PC or W-PC. Two patients suffered from oral bleeding secondary to grade 2 mucositis and grade 1 cutaneous bleeding the day that they were transfused with W-PC. Meanwhile, one patient suffered from cutaneous bleeding (grade 1) and two had oral bleeding secondary to mucositis (grade 2) the day they were transfused with S-PC (Table II).

There were not a statistical difference between the duration of storage of S-PC and W-PC before transfusion (2.6±1.4 days for S-PC *vs* 2.3±0.7 days for W-PC; p=0.56).

Pre- and post-transfusion platelet counts in the patients, platelet count increments and 1 h and 24 h post-transfusion CCI are shown in Table III. The patients platelet counts were similar before transfusion of either S-PC or W-PC (18.5×10^9/L *vs* 16.9×10^9/L, respectively). Overall, we observed a tendency to a slightly reduced transfusion efficacy for W-PC, although the difference only reached statistical significance for the 1 h post-transfusion platelet count (Table III). Both 1 h and 24 h CCI, and the percentages of successful transfusions at 1 h (CCI >7.5×10^9/L) and 24 h (CCI >4.5×10^9/L), were slightly higher with S-PC than with W-PC (Table III).

It is worth noting that none of the patients in this small study had adverse transfusion reactions or septic events after being transfused with either S-PC or W-PC.

Discussion

Transfusion of washed platelets is indicated for patients with previous allergic transfusion reactions or IgA or haptoglobin deficiency. On-demand preparation of this platelet product is hampered by the lack of international recommendations[5,6]. In this study we validated an in-house method to obtain washed platelets for clinical purposes. The procedure is simple, takes only about 2 h, and does not require very specialised personnel. Once validated, the protocol has been easily implemented for its general use by trained technicians in the blood component department of our Blood Transfusion Centre.

Table II - Clinical status and bleeding complications in patients transfused with standard or washed platelets.

	Platelets		p-value
	S-PC	***W-PC***	
Fever			1
No	8 (83)	8 (83)	
Yes	3 (27)	3 (27)	
Diarrhoea			1
No	10 (91)	11 (100)	
Yes	1 (9)	0	
Mucositis			0.68
No	5 (46)	5 (46)	
Grade 1	1 (9)	2 (18)	
Grade 2	1 (9)	0	
Grade 3	2 (18)	4 (36)	
Grade 4	2 (18)	0	
Bleeding			0.81
No	8 (73)	9 (82)	
Yes	3 (27)	2 (18)	
Sepsis			
No	11 (100)	11 (100)	

All values are expressed as percentages (%) from 11 patients. S-PC: standard platelet concentrates; W-PC: washed platelet concentrates.

Table III - Assessment of transfusion efficacy of standard and washed platelets.

Parameter	Platelets		p-value
	S-PC	***W-PC***	
Total platelet transfusions (n)	11	11	
Pre-transfusion platelet count ($\times10^9$/L)	18.5±7.3	16.9±8.7	0.41
1 h post-transfusion platelet count ($\times10^9$/L)	49.1±16.2	42±18	0.02
24 h post-transfusion platelet count ($\times10^9$/L)	44.1±23	36.1±15.5	0.10
1 h PPI ($\times10^9$/L)	30.6±16	24.8±19.5	0.06
24 h PPI ($\times10^9$/L)	25.6±18.9	19.2±16.6	0.14
1 h CCI ($\times10^9$/L)	13.5±5.6	11.5±7.3	0.25
24 h CCI ($\times10^9$/L)	11.1± 7.2	9±6.5	0.24
Transfusions with 1 h CCI >7.5×10^9/L (n, %)	10 (91%)	7 (64%)	0.31
Transfusions with 24 h CCI >4.5×10^9/L (n, %)	10 (91%)	9 (82%)	1.00

Values are expressed as mean ± standard deviation or percentages (%) from 11 patients, as appropriate. S-PC: standard platelet concentrates; W-PC: washed platelet concentrates; PPI: post-transfusion platelet increment; CCI: corrected count increment.

Our platelet washing protocol is performed in a standard blood bank centrifuge, similarly to most methods previously described[14-17]. In contrast, few others have evaluated washed platelets processed with semi-automatic devices, such as a Cobe 2991 (Terumo Bct, Tokyo, Japan) or ACP215 (Haemonetics Co, Braintree, MA, USA)[18-20]. The use of this equipment may require more specialised personnel. Of mention, the COBE 2991 is no longer on the market. Recently, a hollow-fibre column system has been adapted for the preparation of W-PC, with acceptable maintenance of *in vitro* platelet function[21].

A major drawback of platelet washing procedures in a clinical setting is platelet loss. Previous reports of washing platelets by means of a single centrifugation step have shown losses from 4 to 18% of the initial platelets[14,17], while those using a two-step centrifugation registered platelet losses of 26 to 40%[15,18,21-23]. In the present study the mean percentage of recovery for W-PC was as high as 85%, thus being acceptable for transfusion purposes.

Another potential detrimental effect of washing platelets is impairment of platelet function. In our *in vitro* platelet quality assessment we found that washed platelets essentially maintained their aggregability potential, except with mild agonists such as ADP. These findings are in accordance with previous reports showing unaffected aggregation response to collagen in washed platelets, while their aggregation response to ADP or epinephrine was severely impaired[15,16]. While VerifyNow® is currently widely used for the purpose of antiplatelet therapy monitoring[9], this is the first study showing the potential utility of this point-of-care device for evaluating platelet products in a blood bank setting. This device showed that washing platelets had a detrimental effect on the platelets' response to weak agonists, while not interfering with their reactivity towards a potent agonist, thrombin.

In contrast to a few earlier studies in which washing induced extremely marked platelet activation[15,19], this parameter was very mildly affected in our washed platelets. In addition, the exposed P-selectin on the platelet membrane surface upon TRAP stimulation was similar before and after washing, again suggesting a minor effect of washing on thrombin-mediated platelet activation and secretion. It is noteworthy that Rignwald *et al*. also reported a low level of spontaneous activation in washed platelets, despite using a protocol involving two centrifugation steps[15]. As they reported a very high platelet loss during washing, there was concern about whether activated platelets had been selectively lost. Nevertheless, these discrepancies among studies suggest that washing procedure variability (centrifugation steps, washing solutions, etc.) has a profound impact on platelet activation[14-16,19,21].

A major issue in clinical transfusion settings is whether loss of platelets and altered platelet function during product processing would have a significant detrimental effect on transfusion efficacy. Indeed, in the present study, we observed a trend to a slightly higher transfusion efficacy for S-PC transfusions compared to W-PC transfusion, and yet only the 1-h post-transfusion platelet count was statistically significantly different (Table III). Importantly, more than 80% of the W-PC transfusions were defined as effective transfusions at 24 h, and transfusion of washed products was not associated with an increased number of bleeding events. Concordantly with these findings, Azuma *et al*. reported that washed and/or plasma-reduced apheresis platelet products prevented allergic reactions and non-haemolytic reactions in 11 out of 12 patients, provided adequate 1 h and 24 h CCI, and were associated with no clinical bleeding[4]. In contrast, Karafin *et al*. reported major differences, from 32.2 to 50.6%, in 1 h and 24 h CCI between standard and washed apheresis platelets, which may have been due to high platelet loss in their washing protocol and/or to unrecorded complications in the recipients, such as splenomegaly and fever[23].

The design used in this study, two platelet transfusions in the same patient in a short time, was intended to minimise the effect of the patients' clinical status on the transfusion efficacy while maximising the influence of the platelet product itself. Nevertheless, one limitation of this study is that the number of patients evaluated was low and larger series are needed to ensure the clinical efficacy of platelets washed according to our protocol. Moreover, the low prevalence of patients with previous allergic transfusion reactions or IgA or haptoglobin deficiencies make it unfeasible to perform the study in those patient populations for whom washed platelets are most commonly indicated.

Conclusions

In summary, we have implemented a simple process to prepare washed platelets, with a minor impact on platelet recovery and platelet activation and function. A pilot transfusion study supported the clinical haemostatic efficacy of these washed platelets. Future trials in patients with allergic transfusion reactions or IgA or haptoglobin deficit are needed to evaluate the effectiveness of this product at preventing undesirable transfusion reactions mediated by plasma proteins contained in the PC.

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Authorship contributions

JR and HF-M designed the research and wrote the paper. EMP, MJC, MLA, FF-M and JR prepared the platelet concentrates and evaluated their in vitro quality. HF-M, CC-L, CM-M, PI, IH and VV performed the platelet transfusions, recorded and first evaluated clinical parameters. All Authors critically reviewed and approved the final version of the paper.

The Authors declare no conflicts of interest.

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