Original Article

Access this article online
Quick Response Code:

Website: www.ajts.org
DOI: 10.4103/ajts.ajts_145_21

Platelet additive solution suspended apheresis platelets in a tertiary care hospital: A step toward universal single donor platelets

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Abstract:

BACKGROUND: Transfusion of ABO-compatible single donor platelets (SDP) is preferable for better outcomes over group switchover SDP. The use of SDP containing ABO-incompatible plasma is associated with a risk of allergic and acute hemolytic transfusion reactions. Moreover, high titer O group donors SDP impose a further threat to patient safety. Platelet additive solution (PAS) is used worldwide for the storage of platelets which reduces plasma volume available in SDP. SSP + (Macopharma) is one such PAS which can provide improved availability, logistical management, decrease wastage, and improvement in patient safety. The aim of this study was to assess the feasibility of using PAS to obtain low titer SDP units which can be utilized across a larger patient population and to study quality control parameters of these units.

MATERIALS AND METHODS: The study was performed in the department of Transfusion Medicine from June 2017 to January 2018 after clearance from the Institutional Review Board. The study design comprised two cohorts (A and B). In cohort A, the temporal trend of *in-vitro* changes in the quality parameters was tested and analyzed for PAS modified and unmodified products on days 1, 5 and 7. In cohort B, the original plasma from the SDP donors of all blood group donors except the AB group was tested for antibody titers before (prepreparation) and after modification (postpreparation) by PAS.

RESULTS: In cohort A, in the control group, there was a significant change in the mean platelet volume, potassium, and bicarbonate levels from day 1 to day 7, whereas no significant change in the biochemical parameters was noted in the study group where PAS was used. In cohort B, on comparing the anti-A and anti-B, before and after modification of SDP with PAS, there was a significant reduction in the median titers across all the groups studied.

CONCLUSION: PAS added SDP is an efficient strategy to reduce the ABO-antibody levels significantly. PAS added SDP also helps in the better inventory management of available groups.

Keywords:

Modified platelets, platelet additive solution, solid state drive

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Submitted: 25-09-2021
Revised: 14-11-2021
Accepted: 11-12-2021
Published: 26-05-2022

Introduction

The need for blood is rising each day due to advancements in clinical medicine.[1] The availability of safe blood products is a major component in improving health-care sectors.[2] Platelet (PLT) transfusions are one such critical part in managing a wide group of patients.[3]

ABO-identical PLT transfusion is warranted for the safest transfusion strategy but applying this policy to all patients is not always practical due to limited availability of ABO-identical PLTs in emergency care

How to cite this article: Chowdhry M, Agrawal S, Prakash B, Thakur UK, Mishra M. Platelet additive solution suspended apheresis platelets in a tertiary care hospital: A step toward universal single donor platelets. Asian J Transfus Sci 2022;16:238-44.

and limited 5 days' shelf-life.[4,5] Yet, in cases of ABO and/or RhD incompatibility, there are only limited recommendations for PLT transfusions which do recommend ABO group switchover for PLTs is accepted during emergencies.[6-8]

PLTs have on their surface the carbohydrate antigens of the ABO blood group. The plasma obtained from a donor associated with PLT concentrates has naturally occurring antibodies against A or B antigens that are lacking in the donor.[9,10] The accelerated destruction of the PLT in the recipient can occur after a major ABO-incompatible transfusion. The transfusion of PLTs containing high titers of antibodies to the antigens on the patient RBCs may cause clinically significant hemolysis.[4] Although relatively rare, acute hemolytic transfusion reactions (AHTRs) after minor ABO-incompatible PLT transfusion continue to occur in the range of 1: 2000-1: 46,176, depending on whether the evaluation is based on numbers of transfused products or transfusion events.[4,11] Moreover, while high titers of anti-A and anti-B should logically be more strongly associated with the development of symptoms than lower titers, case reports of adults who develop hemolysis due to plasma incompatible apheresis PLT transfusions demonstrate that titers <128 may cause symptoms.[10,4] The current risk of an AHTRs following PLT transfusion with minor ABO-incompatibility is higher due to the increased use of single donor's PLTs, which contain 4–8 times more plasma than random donor's PLTs.[11] Patients receiving PLTs are often critically ill, and symptoms and signs of hemolysis in these patients might not be attributed to PLT transfusion.[12,13] Different blood transfusion services have devised their policies on group switchover concerning PLT transfusion. Such policies include[14] screening PLT donors for high titer ABO antibodies,[4] encourage manufacturers to develop and validate automated anti-A and anti-B titer screening,[13] wash and re-suspend PLTs in saline solution, reduce the plasma volume of Group O apheresis PLTs concentrates to 50 mL,[15] and replace plasma with additive solutions or AB plasma after washing.[16-18] However, these approaches do not completely prevent the risk of HTR.

Recent studies have focused on platelet additive solutions (PAS)-based single donor PLT (SDP). PAS is the generic term for any balanced electrolyte solution used to store low plasma carryover PLTs. It is extensively practised across the world and has gained momentum in India as well. PAS replaces incompatible plasma and makes PLTs switch across groups more scientific. PAS leads to reduction in the number of allergic transfusion reactions apart from reducing the risk of hemolysis due to passive transfer of naturally occurring antibodies. Besides, the extra-recovered plasma may be utilized for fractionation.[19]

The pH of the PLTs suspended in PAS is similar to that of PLTs stored in plasma.[20] Advantages associated with the use of PAS include reduced risk of hemolysis, issue of product to all group patients/recipients, reduction in associated allergic reactions to patients, smooth inventory management, and wastage prevention.[21] To further improve the PLT quality, potassium and magnesium were added to standard PAS to prepare SSP + (PAS-E), which has shown promising results for *in vitro* parameters.[22,23] Main advantages of SSP + over standard PAS are the preservation of PLT function, the compatibility with pathogen inactivation, and the PLT shelf life extension of up to 7 days. However, extended storage may cause progressive and deleterious changes, collectively termed the "platelet storage lesion," which decrease PLT quality *in vitro* and *in vivo*.[24]

This study was done to compare the quality parameters between apheresis PLT containing PAS (SSP+) and apheresis PLT suspended in plasma and to assess the antibody titers before and after modification with PAS.

Materials and Methods

A prospective study involving the production of PAS-modified SDP's (n = 200, consecutive) was carried out in our multispecialty tertiary care hospital after clearance from the Institutional Review Board. The duration of the study was 8 months from June 2017 to January 2018. Screening for apheresis donors was the same as for normal donors. Other basic requirements include PLT count to be more than 150,000/μL, no history of drug ingestion, especially aspirin and presence of good veins that do not collapse under the pressure exerted by the apheresis machine.

The SDP's were prepared using the Amicus apheresis machine (Fenwal). Instead of PLTs being suspended in 100% donor plasma, PASs (SSP+, Macopharma) was added under sterile conditions in the ratio of 70:30 with the plasma. The targeted SDP yield was 4×10^{11}/unit.

The study design comprised two cohorts (A and B) [Figure 1]. In cohort A, the following parameters were tested and compared between the study group and control group on days 1, 5, and 7: the age of the donor, prepreparation PLT count of the donor, visual appearance of the product, swirling, PLT yield. Arterial blood gas analysis (pCO_2, O_2, HCO_3^-, K+) was measured using a blood gas analyzer (ABL800 BASIC) immediately after sampling at 37°C. pH was measured at 22°C. PLT indices (i.e., mean platelet volume [MPV], mean platelet component [MPC) were measured by the Advia 2120i hematology analyzer. Swirling was assessed by visual inspection and graded as Score 0 (no swirling), +, ++, or +++ (maximum swirling). The control group included

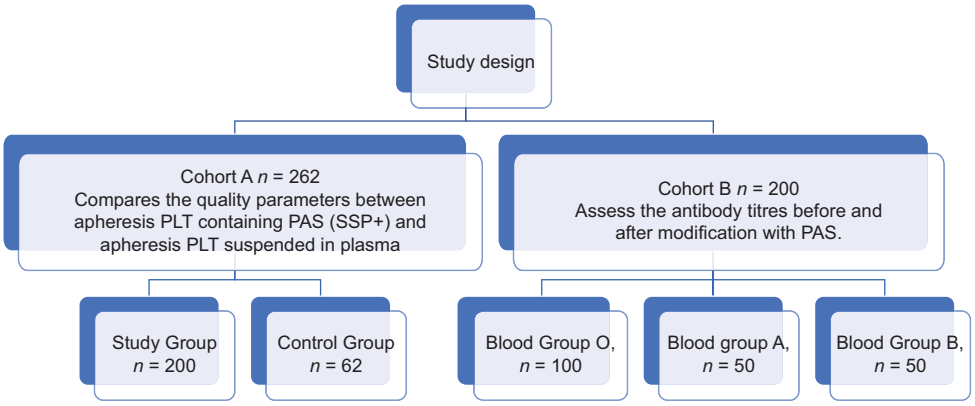

Figure 1: Study design

AB group SDP where PAS was not used for storage. All plasma samples were stored at 4°C until tested.

In cohort B, except for AB group donors, the ABO antibody titers were tested using the donors' sample before making the SDP suspended in PAS and were also tested from the sample obtained after making PAS suspended SDP from these donors and compared. The titers of anti-A and anti-B were studied by tube technique according to the departmental protocol.

"Dangerous O group donors" were defined as O blood group donor plasma with a potential to agglutinate erythrocytes of non-O recipients due to high titers (>128) of anti-A and/or anti-B hemagglutinins In O group SDP's, anti-A and anti-B titers were compared before and after modification of SDP with PAS and between "high titer, i.e., >128" and "low titer group i.e., ≤128."[10] Universal SDPs were stored at 22°C under constant agitation.

Statistical analysis

The temporal trend of *in-vitro* changes in the quality parameters was tested and analyzed for PAS modified and unmodified products on day 1, 5, and 7. The correlation of each parameter was estimated using the log-rank Mantel-Cox test; multivariate analysis was performed using the stepwise Cox proportional hazard regression method. The data were analyzed using (IBM) SPSS version 23. A $P < 0.05$ was considered statistically significant for all tests.

Results

All donors participating in this study met the guidelines for the selection of SDP donors.[10,25] Ninety-seven percentage of donors under the study were male and 58% were in the age group 18–30 years.

Two hundred units of SDP were prepared using PAS as an additive solution during the study period. These included 100 Group O donors and 50 each of A and B group donors. The control cohort group (n = 62) included AB group SDP where PAS was not used for storage.

In study cohort A, quality parameters were tested between the control and the study group [Tables 1 and 2]. Most quality control parameters in the study group viz. the visual appearance of the product, swirling, PLT yield, pH, pCO_2, pO_2, K+ and PLT indices did not change significantly from day 1 to day 5 and day 7 [Table 1]. The bicarbonate levels decreased gradually but were maintained at a level of 2.6 mmol/L, approximately, from day 5–7. The MPV did not change significantly and ranged from 8.9 to 9.0 fl from day 1 to day 7. The pH levels were maintained from day 1 to day 7. Swirling was fully maintained (graded by visual inspection) in all units of the study group. In the control group, there was a significant change in the MPV, potassium, and bicarbonate levels from day 1 to day 7. The MPV value increased from 9.1fl at day 1 to 10.4 fl at day 7. Similarly, the bicarbonate levels decreased from 13 to 1 mmol/L from day 1 to day 7. The potassium levels also increased to 6.5 mmol/l on day 7. All other parameters showed a nonsignificant change over 7 days. Swirling was fully maintained (graded by visual inspection) in all units of the control group [Table 2]. On comparing each parameter of the control and study group using the log-rank Mantel-Cox test, it was observed that serum bicarbonate level was significantly deranged when compared between the two groups on days 5 and 7, and days 1 and 7 ($P < 0.05$). However, comparison on days 1 and 5 showed no clinically significance ($P > 0.05$). All other parameters compared on days 1, 5, and 7 were statistically insignificant. Multivariate analysis showed that on analysing all parameters together between the two groups, no statistical significance was observed ($P > 0.05$). P values for comparisons between Tables 1 and 2.

In study cohort B, in Group O patients, titers were determined in 100 patients. Out of these

100 patients, "high titer" was observed in 48 patients and "low titers" in 52 patients. In these 100 patients, on comparing the anti-A and anti-B titers, before and after modification of SDP with PAS, there was a three, serial two-fold reduction in the median titers ($P < 0.01$) from 256 to 32 in both anti-A and Anti-B in "high titer" group. In "low titer" group as well, the serial reductions of titers were significant with a two, serial two-fold reduction ($P < 0.05$). Similarly, in blood groups A and B, anti-B and anti-A titers showed two serial two-fold and three serial two-fold reductions, respectively [Table 3].

Discussion

PAS are crystalloid nutrient media used to replace the plasma (up to 60%–70%) in PLTs and provide better storage and retention of PLT properties.[3] During the past two decades, there has been an upsurge in the use of PAS for the storage of PLTs.

To optimize the storage conditions, reduction in the PLT activation and inclusion of various components in the PLT storage environment such as citrate, acetate, glucose, potassium, and magnesium are added.[25] The acetate in the PAS solution acts as a substrate for PLT metabolism along with glucose. It also helps to maintain the pH during storage. The phosphate in the PAS solution not only maintains the pH by acting as a buffer but it also helps in maintaining well *in vitro* characteristics during interruption of agitation. Besides the effect on PLT metabolism, many of the components affect the function of the PLT membrane. The citrate present in the solution prevents coagulation; magnesium prevents aggregation of PLTs and causes a reduction in PLT activation. Potassium in the solution prevents aggregation, causes a reduction in PLT activation, causes a reduction in glycolysis and helps in maintaining membrane potential (to avoid leakage of potassium ions) and pH levels.[26,27]

This study essentially investigates two major points, i.e., the comparison of various indices between the study and the control group (Cohort A) and the antibody titers before and after the modification with PAS (Cohort B). Both the cohorts were adequately studied and important information was obtained on analysis. "Swirling phenomenon" is observed when the movement of the suspension causes PLTs with discoid morphology to align their long axis parallel to fluid flow. The swirling represents a rough idea of an intact PLT shape.[28] Swirling score is reduced in case of storage lesions (which occur due to biochemical, physical and functional changes during storage) in the apheresis PLT product as established previously by Feys *et al*. This is also related to an increase in PLT count,

Table 1: Quality control parameters in Cohort A study group (*n*=200)

	Swirling	Visual inspection	Mean							
			PLT count ($\times 10^{11}$)	pH	pCO_2 (mmHg)	pO_2 (mmHg)	MPV (fl)	MPC	Potassium (mmol/L)	Bicarbonate (mmol/L)
Day 1	+++	Clear	3.4	7.0	22	98	8.9	18.2	4	6.1
Day 5	+++	Clear	3.5	6.9	14	110	8.8	17.8	4.5	2.6
Day 7	+++	Clear	3.3	6.9	13	115	9.0	16.8	4.9	2.6

Swirling Strength grades as +,++,+++,++++. PLT=Platelet, MPV=Mean platelet volume, MPC=Mean platelet component

Table 2: Quality control parameters in Cohort A control group (*n*=62)

	Swirling	Visual inspection	Mean							
			PLT count ($\times 10^{11}$)	pH	pCO_2 (mmHg)	pO_2 (mmHg)	MPV (fl)	MPC	Potassium (mmol/L)	Bicarbonate (mmol/L)
Day 1	+++	Clear	3.0	7.2	35	98	9.1	19.9	3.6	13
Day 5	+++	Clear	3.3	6.8	20	112	8.9	18.1	3.8	7.2
Day 7	+++	Clear	3.2	6.5	23	144	10.4	17.1	6.5	1

Swirling Strength grades as +,++,+++,++++. PLT=Platelet, MPV=Mean platelet volume, MPC=Mean platelet component

Table 3: Titers of the donor before donation and postmodification of product in Cohort B

Blood group	*n*	Pretiter median		Posttiter median	
		Anti A	Anti B	Anti A	Anti B
O	100				
	High versus low titer group				
	High (48)	256 (256-1024)	256 (256-1024)	32 (16-128)	32 (16-256)
	Low (52)	128 (4-128)	128 (4-128)	32 (1-32)	32 (1-32)
B	50	32 (2-64)		4 (1-8)	
A	50		16 (2-64)		4 (1-8)

but a reduction in pH and glucose levels (due to higher metabolic activity) and poor integrin response lead to the poor quality/compromise in quality of the product.[29] In our study, the swirling gradation score assessed by visual inspection was adequate in both the study groups indicating good viability and well maintained hemostatic properties during storage till 7 days. These results are in confirmation with previous findings published by Jain *et al*.[30]

The platelet distribution width (PDW) is used as a measure of PLT volume heterogeneity and anisocytosis. PDW is increased in the case of PLT anisocytosis. The normal range of PDW is 8.3%–56.6%. PDW together with MPV gives a more complete description of the PLT volume distribution than MPV alone. The MPV is a marker of both PLT function and activation. It is expressed in femtoliter (fl) with the normal range between 7.2 and 11.7 fl. It is used as a marker of increased production rate and PLT activation.[31] It has been shown that MPV is a reliable measure of residual PLT function in stored platelet concentrates (PC), an increase in MPV indicates the deterioration of the product.[28] It has also been found that the MPV of stored PC is inversely proportional to pH, indicating poor quality of the product.[32] In cohort A of our study (Control and Study group), we did not observe any significant difference/variation in PLT indices like MPC. However, the difference in MPV was observed in the control group. MPV value increased on day 7 in the control group. This can be correlated with low pH and low bicarbonate levels, thereby causing an abruption of morphology implying deteriorating quality of PLTs on day 7 of storage without PAS. Singh *et al*. studied that there exists a strong correlation between pH and PLT indices, for example, difference in MPV, and PDW during storage[32] ($P < 0.01$).

The pH is a detrimental factor, which falls with a rise in metabolic activity of PLTs affecting the storage.[24,33] PAS addition helps in maintaining pH, PLT yield, and count.[33] Reduced pH level <6.2 and more than 7.4 significantly affects the shape (from discoid to spherical or fragmented form) and volume of PLTs which is highly undesirable for transfusion.[27] In our study, the PLT yield/count and pH of the product was in acceptable limits even on the 7th day of storage after the addition of PAS. Our results are in confirmation with previously reported studies demonstrating that PAS-suspended PLTs have improved maintenance of pH, with a reduction in glucose consumption and lactate production.[3,34,35]

In the present study, the bicarbonate levels have also decreased considerably from day 1 to day 7, in both the groups (control and study). However, the decrease was considerable in the control group, wherein no PAS was used. The reduction in bicarbonate is due to its role in providing buffer capacity at the time of anaerobic metabolism of PLTs to maintain the essential adenosine triphosphate levels. Previous studies have also reported a similar trend in observing the PLT quality parameters.[27,30] The pCO_2 decreased and pO_2 slightly increased (insignificant) from day 1 to day 7 in both the control as well as in study groups. This shift in the pressure of gases, further results in a decrease in the lactic acid production, in anaerobic metabolism in PLTs, indicating the good storage of the units.[30,32,36]

The critical titer levels depend on the method adopted and it has been suggested to be >1:16 for *in vitro* hemolysis assay, >1:64–1:100 for direct agglutination, and >1:256–1:400 for indirect antiglobulin test.[30] However, the clinically associating critical titer levels have not been defined clearly anywhere in the available literature.[3,19] A cut-off level in the range of 128–250 is defined as high titer units in most of the blood banks.[37] In our study, a titer of more than 128 was considered for defining the "high titer" group.

In blood group O, high titer was observed in 48% and low titer in 52% of donors. In our study, a significant reduction in serial titers was observed in all groups. The significant reduction in the antibodies titer is due to the dilution effect of PAS as plasma is replaced by isotonic buffered solution. Several studies have reported similar trends suggesting that the addition of PAS to O, A, and B blood groups significantly reduces the antibody titer and associated risk of adverse reactions posttransfusion.[3,38] Jain *et al*., prepared low-titer group "O" SDP using PAS and found significantly reduced titer levels of anti-A and anti-B in the study arm SDPs ($P < 0.001$) as compared to the titer taken from the pilot group with plasma.[30] The median titer dropped from 128 to 16 for anti-A and anti-B antibodies in the study arm while the maximum antibody titer post-PAS addition in any unit was up to 64.[30] The authors suggested that the substitution of plasma with PAS resulted in a concomitant reduction of isoagglutinin levels reducing the risk of adverse reactions. PAS added SDP is an efficient strategy to reduce the ABO-antibody levels significantly, which not only reduces the risk of adverse reactions but also provides better inventory management at the time of urgent requirement, by limiting the wastage of blood units.[39]

Limitations

In Cohort A, some parameters like PLT activation and aggregation were not analyzed due to the paucity of resources. The testing of various other PAS solutions could not be performed. All the results are based on testing with SSP + solution.

Conclusion

The addition of PAS for apheresis PLTs helps in the better inventory management of available groups. PAS added SDP is an efficient strategy to reduce the ABO-antibody titer levels significantly. The addition of PAS also maintains the quality parameters of the PLTs to acceptable limits till day 7 of storage. However, in this regard only, few Indian studies have been conducted so far and larger clinical studies are needed to explore its benefits and extended storage.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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