

Original Research Article

Study and analysis of the coagulation factors and their natural inhibitors levels in apheresis derived platelet concentrate units over a five-day storage period

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ABSTRACT

Background: The possibility of utilizing clotting factors in the plasma phase of apheresis platelet concentrates, as a supplement to the standard FFP transfusion for clotting factor replacement needs to be explored. In this study, it was proposed to assess the effect of storage on clotting factors and inhibitors in stored apheresis platelet concentrates. This would give an insight into the hemostatic potential of the plasma phase of the apheresis platelet concentrates.

Methods: This study was conducted on a sample size of 45 apheresis platelet concentrate units harvested on Amicus cell separator. Basic coagulation workup along with various coagulation factors and their natural inhibitors were studied in the apheresis platelet concentrates on day '0' and day '5' of the collection.

Results: Prothrombin time and activated partial thromboplastin time of the apheresis platelet concentrates was increased on day '5' of the collection but were within the normal range. Fibrinogen, Factor XII, and VWF: Ag showed an increase on day '5' of collection. Protein C, protein S activity, and antithrombin decreased on day '5' of collection. Also, Factors II, VII, IX, X, XI decreased on day '5'. The highest fall in activity was seen in the case of Factors V and VIII. Despite the fall, all the clotting factors were maintained within their normal range.

Conclusions: Although the activity of most of the coagulation factors showed a decrease, it was maintained within their normal range and efficacy. Therefore, a reasonable hemostatic potential of the clotting factors is expected to be maintained in apheresis platelet concentrates after a storage period of five days at room temperature.

Keywords: Apheresis platelet concentrates, Coagulation factors, Natural inhibitors

INTRODUCTION

Normal hemostasis ensures blood to remain in a fluid state in the blood vessels through a tightly regulated process. Several key factors like the endothelial cell lining of the vessel wall, the various plasma clotting factors along with their natural inhibitors and the platelets participate in maintaining the fine physiological balance between hemostasis and thrombosis.¹

Fresh frozen plasma (FFP) refers to the plasma that has been separated by centrifugation from whole blood within 8 hours of its donation and then stored solid at minus 18°C or lower. It can also be obtained by dedicated techniques such as plasmapheresis with no less than 70% of the original clotting factors and at least comparable quantities of the other coagulation factors and their natural inhibitors are present in it.²

Platelets are a type of enucleated blood cells which function in conjunction with the clotting factors, to achieve hemostasis. Platelet concentrates can be prepared by centrifugation of fresh whole blood or with the aid of specialized blood cell separators.^{3,4} Such platelet concentrates are termed as 'single donor platelets' (SDP) or 'apheresis derived platelet concentrate' (APCs) units and contains $3-7 \times 10^{11}$ platelets per unit suspended in 200-300 ml of plasma.

Platelets stored between 20 to 24°C in platelet agitators have a shelf life of five days.⁵ Storage at this temperature is not an ideal environment for preservation of the clotting factors (especially the thermolabile ones), present in the plasma phase of platelet concentrates. Processing and storage of these components cause activation of platelets, thereby contributing to the platelet storage lesions, which in turn, affect various coagulation factors.⁶

The possibility of utilizing clotting factors in the plasma phase of APCs' as a supplement to the standard FFP transfusion needs to be explored, since substantial benefit can be obtained by this supplementation, in ongoing depletion of clotting factor levels in a massive transfusion scenario. It is with this intent that, a few studies have been conducted, reporting the effect of storage at room temperature on the clotting factors and their inhibitors in platelet concentrates.^{7,8} In this study, it was proposed to assess the effect of storage on the clotting factors and their inhibitors in stored the APCs', which would give an insight into the hemostatic potential of the plasma phase of the APCs'.

METHODS

Study type: It was an observational prospective study.

Study place: The study was conducted in a tertiary care hospital in Western Maharashtra. Study period was from January 2018 to January 2020.

Plateletpheresis donor selection criteria: All healthy volunteer donors, who met the Directorate General of Health Services (DGHS), Ministry of Health and Family Welfare, Government of India, and AABB Guidelines and Recommendations for plateletpheresis were included in the study.³

Procedure in detail: Forty-five (45) leukoreduced, APC units were collected on a continuous-flow cell separator, Fenwal Amicus with software version 3.5 (Fenwal Inc, Lake Zurich, IL, USA) using the single needle plateletpheresis kit (PL 2410). They were analyzed for various coagulation parameters during the shelf life of the product i.e. on day '0' and day '5' of collection.

Before the plateletpheresis procedure, blood samples of the potential donors were collected and tested for ABO/Rh typing, irregular antibodies and were screened for mandatory infectious disease markers by FDA approved

methodologies (HIV, HBV, HCV, malaria, and syphilis). Additionally, a full blood count was performed on an automated hematology analyzer (Sysmex KX-21 hematology analyzer, Sysmex Corporation, Japan) especially for haemoglobin (Hb), hematocrit (Hct), and platelet count.

Anticoagulant: blood ratio was maintained at 1:9 so that the same would be mimicked in the collected APCs'. After performing the quality control (QC) of the APCs', a 10 ml representative sample was collected on day '0' from the APC to analyze the baseline values of the parameters.

The tests performed on the samples by a fully automated coagulation analyzer STA COMPACT (Stago, France) were prothrombin time (PT), activated partial thromboplastin time (APTT), Factor II (FII), Factor V (FV), Factor VII (FVII), Factor VIII (FVIII), Factor IX (FIX), Factor X (FX), Factor XI (FXI), Factor XII (FXII), vWF: Ag, protein C activity (PC), fibrinogen (FBG), antithrombin (AT). Free protein S activity estimation was done using ELISA (Asserachrom free protein S kit).

The product bags were then stored in a platelet agitator/incubator at 22-24°C. Another 10 ml representative sample was collected on day '5' of the shelf life of the product and the same coagulation parameters were analyzed once again.

All samples were drawn from the bag aseptically by using sampling-site couplers in a 10 ml disposable syringes, after gently mixing the contents of the bag. It was then transferred slowly into sterile 12×75 mm, polystyrene tubes for laboratory evaluations

Ethical approval

The study was approved by the Institutional Ethical Committee.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics version 22. One-sample Kolmogorov-Smirnov test was applied, to test for normality. Median and interquartile range (IQR) were used when the change in the variable was not normally distributed. Wilcoxon Signed Ranks Test (WSRT) was used to find the statistical significance for these variables. Mean and standard deviation (SD) were used when the change in the variable was normally distributed. A paired t-test was used to find out the statistical significance for these variables.

RESULTS

The study was conducted on 45 APCs'. The average volume of the product in each bag was 239.8 ml (ranging from 205 to 296 ml), whereas the average platelet count per bag was 5.03×10^{11} (ranging from 3.1 to 6.8×10^{11}). All the laboratory parameters were measured on these APCs'

on day '0' i.e. day of collection and on day '5' i.e. on the fifth day of collection. The entire data were analyzed using IBM SPSS Statistics version 22. The results are compiled in Tables 1 and 2.

Table 1: Variables which showed a normal distribution.

Variables	Day '0'		Day '5'		Significance (paired t-test)
	Mean±SD		Mean±SD		
PT (sec.)	13.78±0.76		15.92±0.44		<0.001
APTT (sec.)	34.33±1.90		39.71±2.33		<0.001
PC (%)	118.36±16.03		113.00±13.97		0.004
FII (%)	90.49±12.76		85.16±13.70		<0.001
FV (%)	83.89±2		62.91±3		<0.001
FVII (%)	90.68±5		74.88±7		<0.001
FVIII (%)	94.78±21.81		61.49±14.97		<0.001
FX (%)	100.73±16.63		91.82±17.09		<0.001
FXII (%)	92.80±16.96		96.07±17.68		0.013
vWF:Ag (IU/dl)	100.96±24.23		118.89±27.70		<0.001

Table 2: Variables which did not show a normal distribution.

Variables	Day '0'		Day '5'		Significance (WSRT)
	Median	IQR	Median	IQR	
FBG (mg/dl)	235	8.5	242	9	0.591
AT (%)	96	2.5	94	2	0.002
PS (%)	84	2.07	81.20	1.87	<0.001
FIX (%)	121	9.5	107	8	<0.001
FXI (%)	113	6.5	110	5.5	0.793

*WSRT: Wilcoxon Signed Ranks Test;IQR- Interquartile Range

The PT relatively increased after the 5th days of storage in all, but one APC. The mean PT on day '5' was 15.92 sec, as compared to a mean PT of 13.78 sec on the day '0'. Similarly, the APTT in all the APCs' showed a relative increase after 5 days of storage. The mean APTT on day '5' was 39.71 sec as compared to a mean PT of 34.33 sec on day '0'. The differences in the mean PT on the day '0' and day '5' and between the mean APTT on the day '0' and day '5' were found to be statistically significant (p<0.001).

The median level of FBG increased from 235 mg/dl on the day '0' to 242 mg/dl on day '5' of storage (p=0.591). The median % activity levels of AT decreased marginally from 96 to 94 (%) after storage of five days (p<0.002). The mean % activity levels of PC on the day '0' and day '5' were 118.36% and 113% respectively and the difference between the two was found to be statistically significant (p<0.004). Median Free PS% activity level was found to be decreased i.e. from 84 to 81.20% over the five-day storage period and the difference between the two was also statistically significant (p<0.001).

FII, FV, FVII, and FVIII showed a loss in the activities by 5.89% (85.1% on day '5' versus 90.49% on day '0'), 25% (62.91% on day '5' versus 83.89% on day '0'), 17.42% (74.88% on day '5' versus 90.68% on day '0') and 35.12% (61.49% on day '5' versus 94.78% on day '0') respectively. FV and FVIII showed a maximum decrease in their % activities. The loss of FII, FV, FVII, and FVIII activities was found to be statistically significant (p<0.001). The median % activity level of FIX was 121% on the day '0' of storage when compared to a median of 107% on day '5' of storage of the APCs' (p<0.001).

There was an 8.84% loss in activity (91.82% on day '5' versus 100.73% on day '0') in FX activity over the five-day storage period (p<0.001). The FXI activity remained relatively stable at a median of 113% (day '5') when compared to a median level of 110% on the day '0' (p=0.793). FXII % activity level showed a 3.52% increase from a mean of 92.8% on the day '0' to 96.07% on the day '5' of collection of the APCs'. Similarly, there was an increase in the mean vWF:Ag levels by 17.75% from 100.96 IU/dl on the day '0' to 118.89 IU/dl on the day '5' of the storage period. Increase in the FXII and vWF:Ag levels were found to be statistically significant (p=0.013 and p<0.001 respectively).

DISCUSSION

Use of platelet transfusion has grown substantially over the last few decades due to the advances made in the hematopoietic progenitor cell transplants, intensive chemotherapy for malignancy, cardiovascular surgical procedures and as a vital component of the massive blood transfusion protocol. Since the platelets are suspended in 200-300 ml of plasma, plasma forms an important constituent of the platelet concentrates. It is well known, that the clotting factors in the plasma, function in vivo in an expanse of plasma, but their interaction with the highly concentrated platelets and its efficacy in the restricted amount of plasma in the APCs' is not known. The possibility of utilizing clotting factors in this plasma phase of APCs' as a supplement to the standard FFP transfusion for clotting factor replacement needs to be explored.

Hence, a substantial benefit can be obtained by its supplementation, to correct the ongoing losses of clotting factor levels in cases of dilutional or consumptive coagulopathies, as seen in the case of massive transfusion scenarios.

With this intent, a handful of studies have been conducted across the globe, studying the effect of storage of platelet concentrates at room temperature, on the clotting factors and their inhibitors. In our study, we noted a few significant changes in the clotting factors and their natural inhibitors in the APCs' throughout five days of storage at room temperature.

Basic coagulation workup

Although the PT and APTT of the APCs were increased during the storage period of five days, due to likely fall in coagulation factor activities at room temperature, their levels were well maintained within the normal range. The PT and the APTT represent the extrinsic and the intrinsic pathways of coagulation respectively, therefore it can be deduced that the vital clotting factors have maintained their activity adequately, enough to maintain these two parameters within their normal ranges. Our study corroborated with the findings of two other similar studies conducted by Weiss et al and Cookson et al, wherein these parameters were also prolonged after five days of storage of the APCs at room temperature and remained within the normal ranges.^{7,8}

FBG Content of APCs

FBG levels in the APCs increased over the five-day storage and remained well within the normal range. FBG is an important constituent of platelet α -granules. Hence, this increase in FBG could be attributed to the degranulation of α -granules of the platelets. The study conducted by Weiss et al on the same subject also yielded an increase in the FBG levels after storage of the platelet concentrates over five days.⁷

Natural anticoagulants

There was a marginal, however statistically significant fall in the AT, PC, and Free PS activity levels ($p=0.002$, $p=0.004$, $p<0.001$ respectively) in the APCs after five-day storage, but the levels were maintained in their respective normal ranges. This fall in AT, PC, as well as the Free PS in the APCs, corroborates with the findings of the studies conducted by Weiss et al and Cookson et al.^{7,8} It can be hypothesized that the decrease in PC activity levels over the five-day storage period at room temperature exceeds the replenishment by degranulation of the delta granules of the platelets. PS percentage activity is known to be very labile over time and, since PS acts as a cofactor for the activity of PC, the decrease of Free PS percentage activity levels can be attributed to these reasons.

Factor assays

Factors II, X, and XI showed fall overtime on day '5' of storage of APCs' at room temperature. There was a statistically significant fall in the percentage activity levels of FII and FX ($p<0.001$), which was consistent with the studies conducted by Weiss et al and Cookson et al.^{7,8}

However, there was an increase in the FXI activity levels in the study conducted by Weiss et al, which was attributed to the release of this factor by the platelet granules. The fall in FXI % activity in our study was only minimal and was not statistically significant ($p=0.793$).

The highest fall in percentage activities was seen in cases of the thermolabile factors, i.e. FV and FVIII ($p<0.001$). The decline in FV % activity level was about 25%, whereas the fall in FVIII % activity was approximately 35% at the end of day '5' of storage. Despite the fall, the mean percentage activity levels of FV and FVIII at the end of day '5' were well maintained within their normal ranges. In the study conducted by Weiss et al, FV % activity decreased by 20%, whereas FVIII % activity decreased by 37%.⁷ A decline in percentage activity over time (approximately 17%) ($p<0.001$) was seen in the case of FVII, which again is consistent with the findings of the study conducted by Weiss et al, although residual FVII % activity at the end of the five day storage period was well within the normal range. FIX percentage activity also decreased significantly ($p<0.001$) over time by around 12% after the 5-day storage at room temperature. The decrease in FIX % activity in studies conducted by Weiss et al and Cookson et al were approximately 8% and 30% respectively.^{7,8} It was observed that although the percentage activity levels of FII, FV, FVII, FVIII, FIX, FX, and FXI in the APCs were decreased over five days of storage at room temperature, they were still maintained within the normal ranges. Hence, although the decrease in the activity of these factors was statistically significant (except FXI), the percentage factor activities in the plasma phase of APCs would have adequate efficacies, to act as a supplement to transfused FFPs', in patients requiring massive transfusion.

There was a statistically significant ($p=0.013$) increase in FXII % activity levels in the APCs' during the storage period of five days at room temperature. FXII is found within α -granules of the platelets. Hence, this increase could be attributed to the release of FXII from the platelet α -granules, after the platelet activation. Similarly, a statistically significant ($p<0.001$) increase in the vWF:Ag levels, during the five-day storage of APCs' at room temperature, could be ascribed to its release from the platelet alpha granules.

It is reiterated that although percentage activity levels of most of the clotting factors showed a decreasing trend, they were maintained well within their normal ranges and efficacies. Therefore, a reasonable hemostatic potential of the clotting factors is expected to be maintained in the APCs after a storage period of five days at room temperature. The hemostatic potential of the plasma phase of the APCs can supplement the activities of coagulation factors provided in the FFP's, transfused as an essential component of the massive transfusion protocol. However, an accurate clinical and laboratory assessment of the hemostatic potential of the plasma phase of the APCs, as a supplement in clinical situations requiring massive transfusion is yet to be studied.

One probable limitation of this study could be the non-availability of multimer-analysis of the vWF, since it is known that the vWF released from the platelets has additional multimers of high molecular weight, compared

to the plasma vWF. This could perhaps have helped in better characterization of the vWF present in the APCs.

CONCLUSION

The adequate hemostatic potential seems to be maintained in the APCs' on their fifth day of storage. These coagulation factors could be of use in achieving hemostasis in massively transfused patients, in whom packed red cells, platelet concentrates, and FFP are transfused in a pre-defined ratio. In such scenarios, this could perhaps redefine the ratios in which these blood and blood components were being used earlier.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethical Committee

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