Original Article

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Assessing Changes in Platelet and White Blood Cell Counts and the Risk of Bacterial Contamination during Storage of Single Donor Platelets: A Cross-sectional Study

Transfusion Medicine Section

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ABSTRACT

Introduction: Single Donor Platelets (SDP) is crucial in managing thrombocytopenia and other platelet-related disorders. Compared with random donor platelets, SDPs minimise donor exposure and reduce the risk of alloimmunisation. Although SDPs are preferred over random donor platelets, their quality deteriorates during storage due to metabolic, biochemical, and morphological changes. These changes can influence post-transfusion platelet recovery and clinical efficacy. Moreover, the risk of bacterial contamination increases with longer storage duration despite leucoreduction and improved storage conditions. Therefore, continuous evaluation of in-vitro quality parameters and post-transfusion outcomes is essential to ensure that stored SDP units remain effective and safe for patient use.

Aim: To assess changes in platelet and White Blood Cell (WBC) counts and the risk of bacterial contamination during storage of SDP, and to evaluate post-transfusion platelet count increment in recipients.

Materials and Methods: The present prospective observational cross-sectional study was conducted in the Department of Immunohematology and Blood Transfusion (IHBT), Nizam's Institute of Medical Sciences, Hyderabad, Telangana State, India, from April 2023 to March 2024. One hundred SDP donors and 100 transfusion recipients were included (patients). The collected platelets were stored in sterile, oxygen permeable polyvinyl chloride bags. A 5 mL sample was drawn on day 0 (day of collection) for WBC and platelet count estimation and for bacterial culture using BACT/ALERT PF+ culture bottles. Comparisons of WBC and platelet counts between day 0 and subsequent storage days (days 1-5) were performed using the paired t-test. Bacterial contamination was assessed by sterility testing (culture reports) from samples collected on each storage day. For evaluating platelet count increments in patients, mean pretransfusion and post-transfusion platelet counts were calculated and compared using the paired t-test. Microsoft Excel was used for data entry, and statistical analysis was performed with IBM Statistical Package for Social Sciences (SPSS) software, version 2022.

Results: The mean donor age was 29.7 years, with an average height of 168.9 cm and weight of 75.5 kg. The most common patient diagnoses were AML (18 cases), followed by CML (14 cases) and lymphoma (15 cases); refractory ITP and cholangitis with severe anaemia were least common (1 case each). Platelet counts showed no significant change on days 0 and 3, but significant reductions were observed on days 1, 2, 4, and 5. Bacterial contamination was detected in some units by day 3 and day 5. There was a significant increase in the patient platelet count ($p<0.05$).

Conclusion: Platelet counts in SDP units decline with longer storage, and patients transfused earlier experience better count recovery. Bacterial contamination risk increases after day 3.

Keywords: Apheresis, Leukoreduction, Microbial detection systems, Platelet count, Platelet transfusion, Plateletpheresis

INTRODUCTION

Platelets are produced through the regulated fragmentation of the cytoplasm of megakaryocytes, the large precursor cells in the bone marrow. Thrombopoietin (TPO), primarily synthesised in the liver, serves as the physiological regulator of platelet production by promoting both the proliferation and maturation of megakaryocytes [1]. Platelets play a critical role in haemostasis, including adhesion to exposed sub endothelium, aggregation at sites of vascular injury, and fibrin-mediated stabilisation of the platelet plug [1].

The SDP are designed to provide a complete therapeutic dose for an adult from a single donor in one collection session [1]. Indications for SDP include amegakaryocytic thrombocytopenia (e.g., leukaemia, aplastic anaemia, chemotherapy, bone marrow transplant, marrow infiltration such as carcinoma or leukaemia, drug or radiation induced hypoplasia), dilutional thrombocytopenia (e.g., massive transfusion), Disseminated Intravascular Coagulation (DIC), and viral infections associated with thrombocytopenia such as dengue [2].

Plateletpheresis, the process of selectively removing platelets while returning red cells, white cells, and plasma to the donor, enables the collection of an adequate therapeutic dose from a single donor [1,2]. This reduces patient exposure to multiple donors, thereby lowering the risk of alloimmunisation and refractoriness [1,2]. Furthermore, SDP offers a lower risk of transfusion-transmissible infections compared with random donor platelets or platelet concentrates [2]. Platelets obtained via apheresis are stored at 22°C in a platelet agitator-incubator for up to five days, with the day of collection designated as day zero [2].

The present study aimed to evaluate the WBC and platelet counts, as well as the risk of bacterial contamination, with increasing storage duration of SDP. Additionally, to assess the post-transfusion platelet count increment in patients receiving SDP transfusion either on the day of collection or after subsequent storage days. The study objectives were twofold: 1) to analyse in-vitro changes in quality parameters of stored SDPs, and 2) to evaluate the in-vivo response to transfusion in recipients based on the storage age of the platelet units.

MATERIALS AND METHODS

The present prospective observational cross-sectional study was conducted in the Department of Immunohaematology and Blood Transfusion (IHBT), Nizam's Institute of Medical Sciences (NIMS), Hyderabad, Telangana, India, from 1st April 2023 to 31st March 2024. The study included 100 SDP donors and 100 patients receiving SDP transfusions at our institution.

Inclusion criteria:

- Adults with platelet counts ≤10 × 10^3/cu.mm to reduce the risk of spontaneous bleeding.
- Patients undergoing elective central venous catheter placement with platelet counts <20 × 10^3/cmm.
- Patients undergoing elective diagnostic lumbar puncture or major elective non-neuraxial surgery with platelet counts <50 × 10^3/cmm.
- Non-thrombocytopenic patients undergoing cardiac surgery with cardiopulmonary bypass.
- Patients undergoing bypass surgery who developed perioperative bleeding with thrombocytopenia and/or platelet dysfunction.
- Patients on antiplatelet therapy with intracranial haemorrhage (traumatic or spontaneous).
- Thrombocytopenic patients' post-chemotherapy or Hematopoietic Progenitor Cell Transplantation (HPCT) receiving prophylactic transfusion to prevent spontaneous bleeding.

Exclusion criteria:

- Patients with a history of allergic or febrile transfusion reactions to platelet products.
- Patients with platelet transfusion refractoriness due to alloimmunisation or HLA antibodies.
- Patients receiving massive transfusion or multi-component transfusions (RBCs, plasma, cryoprecipitate) concurrently with SDP that could interfere with post-transfusion platelet increment assessment.
- Patients with ongoing sepsis, DIC, or splenomegaly, as these conditions can cause excessive platelet consumption or sequestration.
- Pediatric patients (<18 years), as only adult recipients were included.
- Patients with incomplete pre or post-transfusion platelet count data or missing clinical records.
- SDP units showing visible clumping, discoloration, or positive bacterial culture prior to transfusion.
- Recipients who had received antiplatelet or cytotoxic drugs within 24 hours prior to transfusion that could affect platelet survival or recovery.
- All donors were screened using a questionnaire based on the drugs and cosmetics act [3].

Study Procedure

Plateletpheresis was performed using the haemonetics Multicomponent Collection System (MCS+) cell separator, which is automated and requires minimal operator intervention. The system employs intermittent flow through a single-needle access. Just before the start of the procedure chewable calcium tablet was given. Blood was withdrawn from the donor via an apheresis needle and passed through sterile tubing into the machine, where platelets were separated by differential centrifugation. Acid Citrate Dextrose (ACD) to blood ratio was 1:10. At the end of collection, the remaining blood in the circuit was returned to the donor, total volume of platelet volume collected was 250 ml for all the donors [4-6].

The collected platelets were stored in sterile, oxygen permeable polyvinyl chloride bags. A 5 mL sample was drawn on day 0 (day of collection) for WBC and platelet count estimation and for bacterial culture using BACT/ALERT PF+ culture bottles. On subsequent storage days (days 1-5), additional 5 mL samples were taken for repeat WBC and platelet counts, as well as bacterial culture.

Data collection: The study was initiated after approval from the Institutional Ethics Committee with number EC/NIMS/3143/2023. Eligible donors were informed about the study and enrolled after obtaining written consent. Donor data were collected under the following categories:

- **Demographics:** Name, age, sex, height, weight;
- **Patient details:** Clinical history, medication history, past history (blood transfusion, pregnancy/stillbirth/abortion/menstruation, surgery);
- **Clinical assessment:** General well-being and vital signs (blood pressure, pulse);
- **Laboratory investigations:** Complete blood count (haemoglobin, haematocrit, WBC, platelet count) using an automated cell counter, and screening for transfusion-transmissible infections {Human Immunodeficiency Virus (HIV), Hepatitis B surface Antigen (HBsAg), Hepatitis C Virus (HCV)} using Abbott architect with cut-off <1 considered as non-reactive. Screening for malaria and syphilis was done by rapid kits. Haemoglobin>12.5 gm/dL, haematocrit between 40% and 52%, WBC count <11,000/microlitre and platelet count above 2,00,000 per microlitre were accepted.

STATISTICAL ANALYSIS

Comparisons of WBC and platelet counts between day 0 and subsequent storage days (days 1-5) were performed using the paired t-test. Bacterial contamination was assessed by sterility testing (culture reports) from samples collected on each storage day. For evaluating platelet count increments in patients, mean with standard deviation, 95% confidence interval of pretransfusion and post-transfusion platelet counts were calculated and compared using the paired t-test. Microsoft Excel was used for data entry, and statistical analysis was performed with IBM SPSS software, version 2022.

RESULTS

A total of 100 SDP donors were enrolled in the study. The age distribution revealed that more than half of the donors. i.e.,52 donors (52%) were between 18-28 years, followed by 40 donors (40%) in the 29-38 year group, 6 donors (6%) in the 39-48 year group, and only 2 donors (2%) between 49 and <60 years. The mean donor age was 29.7 years, indicating that most donors belonged to the younger adult population, which is considered optimal for plateletpheresis.

The average donor height and weight were 168.9 cm and 75.5 kg, respectively, suggesting a healthy donor pool with adequate physical parameters for safe apheresis donation. Among the total donors, 77% were repeat SDP donors (77 donors), while 23% were first-time donors (23 donors), reflecting a strong base of experienced volunteer donors.

Regarding blood group distribution, the majority were O positive (39%), followed by B positive (34%), A positive (16%), and AB positive (10%). No Rh-negative donors were recorded during the study period. All donors were screened and found negative for HBsAg, HCV, HIV, malaria parasite, and syphilis before donation, ensuring the safety of collected products.

Baseline haematological parameters before donation in SDP donors are summarised in [Table/Fig-1].

Among SDP donors, 72 had haemoglobin levels between 14.6-16.5 g/dL, 11 donors were in the range of 12.5-14.5 g/dL, and 17 donors had levels between 16.6-18 g/dL. Among the 100 patients who received SDP transfusions, the majority 38 patient (38%) were

Parameters	Mean	Range
Haemoglobin	15.5 g/dL	12.6-17.4 g/dL
Haematocrit	44.5%	37.3-49.9%
Platelet	2.8×103/µL	2.06-4.0×103/µL
WBC	6.7×103/µL	3.5-9.8×103/µL

[Table/Fig-1]: Haematological parameters among SDP donors.

in the 18-30 year age group, followed by 27 patients (27%) between 31-45 years, 21% (21 patients) between 46-60 years, and 14% above 60 years (14 patients). The mean patient age was 42.9 years, indicating that most transfusion recipients were middle-aged adults. Of the total recipients, 68 were male (68%) and 32 were female (32%), showing a male predominance in SDP utilisation. A history of previous SDP transfusion was documented in 86 patients (86%), whereas 14 patients (14%) received SDP for the first time during this study period. The diagnosis of all patients receiving SDP are given in [Table/Fig-2].

Diagnosis	Number of patients (n=100)
Acute myeloid leukaemia	18
Chronic myeloid leukaemia	15
Lymphoma	14
Aplastic anaemia	12
B-Acute Lymphoblastic Leukaemia (B-ALL)	10
Acute lymphoblastic leukaemia with allogenic bone marrow transplantation	5
Chronic Lymphocytic Leukaemia (CLL)	4
Carcinoma stomach/rectum	3
Multiple myeloma	3
Liver transplant	3
Thrombocytopenia with bleeding manifestation	2
Splenectomy	2
Pancreatitis	2
Non-cirrhotic portal fibrosis and extra-hepatic portal venous obstruction with hypersplenism	2
Cholecystitis with chronic liver disease	2
Refractory idiopathic thrombocytopenic purpura	1
Cholangitis with severe anaemia	1
Acute febrile illness with thrombocytopenia	1

[Table/Fig-2]: Diagnosis of patient who received SDP.

The highest number of SDP transfusions was administered in the medical oncology (45 patients; 45%), followed by surgical gastroenterology (28 patients; 28%) and the stem cell transplant unit (15 patients; 15%). The remaining 12 patients (12%) received transfusions across other departments, including haematology, hepatology, and critical care.

Among the recipients, 31 patients received only one unit of SDP, 11 patients received between 2-4 units, and seven patients received more than five units.

Blood group distribution showed that patients with B Positive received the highest number of SDP transfusions, followed by O Positive, A Positive, and AB Positive. No transfusions were recorded in patients with Rh-negative blood groups. Haematological parameters of patients receiving SDP transfusions are summarised in [Table/Fig-3].

The paired t-test was applied to compare platelet counts in SDP units between the day of collection (Day 0) and each subsequent storage day (Days 1-5) to evaluate storage-related changes in platelet concentration. As shown in [Table/Fig-4], the difference in platelet counts between day 0 and day 3 was statistically insignificant ($p=0.061$), whereas significant reductions were observed on days 1, 2, 4, and 5 ($p<0.05$). This indicates a progressive decline in platelet counts with increasing storage duration [Table/Fig-4].

Parameters	Mean	Range
Haemoglobin	8.6 g/dL	3.4-14 g/dL
Haematocrit	25.1%	9.7-41.3%
Platelet	19840/µL	5000-70000/µL
WBC	3.6×103/µL	0-27.1×103/µL

[Table/Fig-3]: Haematological parameters among patient receiving transfusions.

Paired t-test		Mean	Std. deviation	95% Confidence Interval of the difference		t	p-value
				Lower	Upper		
1	Day 0	0.02957	0.10958	-0.00298	0.06211	1.830	0.074
2	Day 1	0.11923	0.11869	0.07129	0.16717	5.122	0.0001
3	Day 2	0.18375	0.14362	0.06368	0.30382	3.619	0.009
4	Day 3	0.17750	0.12121	-0.01537	0.37037	2.929	.061
5	Day 4	0.27833	0.16786	0.10218	0.45449	4.062	.010
6	Day 5	0.35500	0.30266	0.13849	0.57151	3.709	.005

[Table/Fig-4]: Paired t-test comparing platelet counts in Single Donor Platelet (SDP) units between the Day Of Extraction (DOE) (Day 0) and subsequent storage days (Days 1–5).
*p<0.05 is considered significant.

As observed in [Table/Fig-5] the increment in platelet count is significant as p-value is less than 0.05 on day 0, day 1, day 2 whereas it is insignificant on day 3, day 4 and day 5.

Storage day of SDP	No. of patients	Mean platelet count (before transfusion) (/µL)	Mean platelet count (after Transfusion) (/µL)	Mean difference (/µL)	p-value	Significance
Day 0	46	18,239	37,630	+19,391	0.000	Significant
Day 1	26	22,962	35,923	+12,961	0.009	Significant
Day 2	8	17,250	34,000	+16,750	0.024	Significant
Day 3	4	13,750	85,500	+71,750	0.268	Not significant
Day 4	6	17,667	52,000	+34,333	0.181	Not significant
Day 5	10	24,900	62,000	+37,100	0.247	Not significant

[Table/Fig-5]: Paired t-test showing day wise comparison in patient platelet count before and after transfusion.
*betx- Before Transfusion *aftx- After Transfusion

The paired t-test was used to compare patient platelet counts before and after SDP transfusion for each storage day group (Day 0-Day 5). A significant increment ($p<0.05$) was observed in patients transfused with freshly collected SDP units (Days 0-2), whereas increments became statistically insignificant for transfusions using units stored for ≥3 days. This demonstrates a decline in post-transfusion efficacy with increasing storage duration.

The median storage duration of SDP units was one day. The mean volume of platelet concentrate collected per donor was 252.5 mL. The mean platelet count in the SDP bag on the DOE was 3.27×10^{11}, which slightly decreased to 3.10×10^{11} on the day of issue, indicating minimal loss of platelet yield during short-term storage.

All SDP units were leucoreduced, and no WBC contamination was detected. BACT/ALERT cultures were performed two hours after collection and again on the day of issue. Out of 100 SDP units, two showed bacterial growth on the day of issue: one with *Staphylococcus epidermidis* after three days of storage and the other with *Aeromonas* species after five days of storage. Both affected recipients were already on antibiotic coverage, and no transfusion reactions were reported [Table/Fig-6].

S. No.	Study ID	Date of collection	Date of issue	Storage duration (days)	Culture result (day of collection)	Culture result (day of issue)	Organism isolated
1	2	8/4/2023	11/4/2023	3	Sterile	Positive	*Staphylococcus epidermidis*
2	3	8/4/2023	13/4/2023	5	Sterile	Positive	*Aeromonas* species

[Table/Fig-6]: Bacterial culture results of SDP units.

DISCUSSION

Platelet transfusion is an essential resuscitative therapy that prevents or controls bleeding in patients with low platelet counts or functional platelet disorders. The decision to transfuse platelets is guided by threshold values that vary depending on the clinical scenario. Although platelet transfusion significantly improves patient outcomes, it is not without risks, including in blood banking and screening techniques, these risks have been greatly reduced [2].

The collection, processing, and transfusion of platelets require strict precision to ensure product quality. Platelet concentrates may be prepared either from whole blood or by apheresis. For prophylactic therapy in adults, the standard dose is 4-6 units of whole blood-derived platelets, which is equivalent to one unit of apheresis platelets. Apheresis platelets are preferred as they minimise donor exposure and reduce the risk of alloimmunisation, since a therapeutic dose is obtained from a single donor [1,2].

In the present study, 100 donors were enrolled over one year. This is comparable to the study by Geeta C et al., (100 donors over 16 months), smaller than those by Singh P et al., (1,472 donors over 5 years) and Sharma R et al., (600 donors over 6 months), but larger than that of Swarup D et al., (40 donors) [4-8]. Similar to previous studies, all donors in the present study were male [Table/Fig-7] [4-6,8].

Study	Year of study	Sample size	Age group	Weight	Platelet count µL	Mean Hb
Swarup D et al., [4]	2009	40	20-49 years	-	1.5-6.25×103	-
Geeta C et al., [5]	2017	100	21-30 years	72.9 Kg	2.0-4.0×103	15.5 g/dL
Singh P et al., [6]	2024	1472	18-40 years	-	-	-
Sharma R et al., [8]	2022	600	18-40 years	73.39 kg	-	15.01 g/dL
Current study	2024	100	18-38 years	75.49 kg	2.06-4.0×103	15.5 g/dL

[Table/Fig-7]: Comparing donor demographic parameters among various studies [4-6,8].

A key finding of the present study was that platelet counts in SDP units decreased progressively with longer storage, which also affected post-transfusion platelet increments in patients. Statistical comparison of platelet counts between the DOE and subsequent Days Of Issue (DOI) showed significant results ($p<0.005$) on Days 1, 2, 4, and 5. On Day 3, results were not significant, likely due to the smaller sample size (only four bags). No WBC contamination was observed, as all SDPs were leucoreduced using the inbuilt filter in the SDP kit.

Analysis of patient platelet count increments after transfusion showed significant improvement on days 0, 1, and 2, but not on later days. This supports the observation that platelet viability and recovery decline with increased storage duration [9-11]. However, factors such as underlying diagnosis and overall patient condition may also influence post-transfusion increments.

Two SDP bags tested positive on culture. One grew *Staphylococcus epidermidis* and the other grew *Aeromonas* species. The results were obtained on the fourth day after issue, and treating physicians were informed. No transfusion reactions occurred, as both patients were already on antibiotic coverage. The *S. epidermidis* contamination was likely due to inadequate cleaning of the venipuncture site, while the *Aeromonas* contamination may have been related to an undiagnosed gastrointestinal problem in the donor. Both cases were noted on days 3 and 5, when the platelet units were close to expiry.

The present study findings are consistent with recent literature indicating that donor biological characteristics significantly influence platelet concentrate quality and storage behaviour. Studies have shown that in-vitro platelet storage properties are affected by donor demographics and haematological parameters, underscoring the importance of maintaining a healthy donor pool for apheresis collections [12]. Furthermore, recent work demonstrated that cytokine levels and activation markers increase with donor age in single-donor platelet concentrates, suggesting that age-related changes can impact product quality [13]. The progressive decline in platelet count and function during storage observed in the current study aligns with contemporary evidence highlighting that platelet viability and haemostatic efficacy decrease over time at room temperature, prompting renewed interest in modified storage conditions such as cold-stored platelets [14]. While the present study contributes valuable data on in-vitro quality and in-vivo platelet increment patterns, the current evidence base remains limited regarding the cost-effectiveness and operational outcomes of different platelet preparation and storage strategies [15]. Finally, a recent multicenter study developed a nomogram model to predict the therapeutic efficacy of apheresis platelet transfusions, emphasising the need for integrated assessment of donor, product, and recipient variables [16]. These findings collectively support the study's recommendation for multicentric, longitudinal studies that correlate biochemical storage changes with clinical transfusion outcomes to optimise platelet utilisation and safety.

Limitation(s)

Being a single-centre, one-year study, the results may not be generalisable to all settings. Continuous bacterial monitoring and biochemical quality parameters were not included. Clinical outcome measures such as corrected count increment and bleeding control were beyond the scope of this analysis.

CONCLUSION(S)

In the current study, it has been observed that the platelet count is decreasing in the SDP bag as the days of storage are increasing. In the patients as we transfused in the initial days the increment in patient's platelet count is significant as compared to transfusion when SDP bag is near to expiry. It has also been observed that as the days of storage increasing bacterial contamination observed in the initial bag on the day of issue i.e., on day 3 and day 5. Future multicentric and longitudinal studies assessing biochemical quality parameters, bacterial kinetics, and clinical outcomes such as CCI and bleeding response are recommended. Advanced sterility monitoring and comparative evaluation of different apheresis systems could further enhance platelet storage safety and efficacy.

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