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ORIGINAL ARTICLE

Changes in Hematological Indices and Lymphocyte Subsets in Response to Platelet Apheresis Donation

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SUMMARY

Background: **Platelet apheresis is a technique in which whole blood is collected from a donor followed by platelet (PLT) separation. Platelet apheresis has a significant impact on some biochemical indices after donation. This study aimed to investigate the impact of platelet apheresis on complete blood count (CBC) and lymphocyte subsets over a typical inter-donation interval.**
Methods: **Healthy male subjects (n = 10) were recruited to study changes in CBC and lymphocyte subsets before and at three intervals following platelet apheresis. Repeated measures ANOVA was used to compare quantitative variables between different visits.**
Results: **Following platelet apheresis, platelet count decreased 30% at 24 hours after donation ($p < 0.001$) compared to the baseline count with significant repeated ANOVA across different visits ($p < 0.001$, Eta = 0.558). No changes were observed in other variables of CBC. The lymphocyte subsets including CD4, CD8, and CD4/CD8 ratio were decreased at 24 hours after donation (-0.6%, -0.4% and -0.7%, respectively) but none was significant. At 24 hours, the proportion of CD19 and CD16-56 were slightly increased (1.6%, 3.3%, $p > 0.05$, respectively).**
Conclusions: **The significant reduction in PLT count after 24 hours of plateletpheresis may have adverse health effects on PLT donors. Platelet apheresis has no significant effect on lymphocyte subsets of the donor.**
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INTRODUCTION

Platelet apheresis is a technique in which whole blood is collected from a donor followed by platelet (PLT) separation. Other blood components including almost all red blood cells (RBC) and white blood cells (WBC) can then be returned to the donor. In 1988, the Food and Drug Administration (FDA) guidelines indicated that the total number of platelet apheresis procedures could be revised to a maximum of 24 platelet apheresis procedures in a year [1]. Therefore, the potential for possible

adverse effects on the donor need to be determined. Recovery of the blood PLT concentration and recovery of other hematological indices are the limiting factors for inter-donation intervals. Thokala et al. showed that the blood PLT concentration recovered after 7 days following platelet apheresis [2]. Other studies have addressed the changes in hematological indices immediately after platelet apheresis [3,4].

Previous studies of changes in immune cell counts following platelet apheresis were undertaken using equipment, that is no longer in routine use [5]. In 1997, Lewis investigated the immune cell counts using new platelet apheresis equipment [6]. We thought it was important to conduct a study of peripheral blood immune cell changes in platelet apheresis donors by using the recent technology of cell separators.

Most of the previous studies have investigated the complete blood cell count (CBC) and lymphocyte subsets immediately after platelet apheresis [3,6,7]. In this study we aimed to assess the effect of a single platelet apheresis donation on CBC indices in a longer-term follow-up and changes to the immune cell count using typical inter-donation intervals.

MATERIALS AND METHODS

Subjects

In total, 10 healthy male donors were recruited. Exclusion criteria were current acute illness, cancer, hematological disease, previous acute myocardial infarction, symptoms of cardiovascular disease, malabsorption, intestinal obstruction or any other condition contraindicating blood donation. Subjects were also excluded if they were being treated with prescribed medications or nutritional supplements such as iron or if fewer than six months had elapsed since a previous blood or platelet apheresis donation. All subjects gave written informed consent for participation. The study was approved by KAIMRC Ethical Committee (Study number RCJ0611-183). A standard donor questionnaire was used to verify eligibility for platelet donation, and a pre-procedure CBC was done to ensure that the donor platelet count was > 150,00 per mm³, with donor consent being obtained before donation as usual.

Study protocol

Measurement of weight and height were taken on each visit for each volunteer. Each volunteer attended the research center at 09:00 on four occasions. At the first attendance (visit A), suitability for participation was assessed and body mass index (BMI) and blood pressure (BP) measured. A blood specimen (10 mL) was collected, by standard venesection technique, into potassium ethylenediaminetetraacetic acid (K-EDTA) for measurement of complete blood count (CBC), screening for abnormal Hb variants, and analysis by flow cytometry (FC). Each subject also had their blood pressure measured using an automated device (V100 DINAMAP, General Electric) before blood collection. Platelet apheresis was undertaken later the same day using a standard protocol at the apheresis facility. The platelet apheresis was performed over a period of 60 to 90 minutes, utilizing the Trima Accel, "Automated Blood Collection System", (manufactured by TerumoBCT, Lakewood, Co., USA), at the blood bank (apheresis area), Department of Pathology and Laboratory Sciences, King Abdulaziz Medical City, Jeddah.

All donation processes were undertaken according to the College of American Pathologists (CAP) and American Association of Blood Bank (AABB) regulations. Visit B was scheduled at 24 hours following platelet apheresis.

Assays and techniques

Complete blood count analyses were carried out using an Advia 2120i analyzer (Siemens, Munich, Germany). Flow cytometry (FC) measurements were performed on a FACSCanto™ II flow cytometer with incorporated Cell QuestTM software for data analysis (BD Biosciences, San Jose, CA, USA). This instrument uses a reagent containing fluorochrome-labelled antibodies which bind specifically to leukocyte surface antigens, staining the cells. RBCs were then lysed and the stained cells measured. Labelling of cells for direct immunofluorescence was performed using monoclonal antibodies (TBNK reagent, BD Biosciences, San Jose, CA, USA). Cells were detected using the antigenic CD3, CD4, CD8, CD19, and CD16-56 cell surface markers. All analyses were carried out in a laboratory fully accredited by both CAP and AABB.

Statistics

Before statistical analysis was carried out, the distribution of data and homogeneity of variances were evaluated using Levene's test. Data were log transformed if necessary. Comparisons of baseline data and data obtained following blood donation were carried out by paired *t*-test. Repeated measures ANOVA was performed as a separate analysis when necessary. Eta squared was used in the ANOVA model to measure the effect size of blood donation on each variable. In all cases, $p < 0.05$ was taken as the level of statistical significance. Results are given as mean ± SEM unless otherwise stated.

RESULTS

Subject characteristics

The age of the 10 included male subjects ranged from 23 to 54 years, with mean ± SEM being 36 ± 1.1 years. Their mean ± SEM body mass index was 27.3 ± 0.5 kg/m^2 (range 20.8 - 35.2 kg/m^2). None of the subjects experienced symptoms or acute adverse effects, such as significant decrease in BP, following donation. None of the subjects had abnormal Hb variants.

Table 1. Complete blood count (mean ± SEM) in healthy subjects pre- and (24 hours, 8 days and 22 days) post-donation.

	Unit	Pre-donation	24 hours	Diff %	8 days	Diff %	22 days	Diff %	Repeated ANOVA	
									Sig	Eta
WBC	x 10^9/L	6.9 ± 0.6	7.4 ± 1.1	6.3	6.9 ± 0.6	0.0	6.4 ± 0.5	-7.2	0.511	0.065
RBC	x 10^{12}/L	5.58 ± 0.24	5.35 ± 0.15	-4.1	5.31 ± 0.14	-4.8	5.26 ± 0.13	-5.7	0.290	0.127
Hb	g/L	15.5 ± 0.4	15.5 ± 0.4	0.2	15.1 ± 0.4	-2.4	15.2 ± 0.3	-1.7	0.212	0.152
Hct	%	46.7 ± 1.06	45.96 ± 1.26	-1.6	45.18 ± 1.06	-3.3	45.33 ± 0.87	-2.9	0.273	0.133
MCV	fL	84.36 ± 2.04	85.96 ± 0.83	1.9	85.2 ± 1.23	1.0	86.39 ± 1.11	2.4	0.377	0.095
MCH	pg	28.01 ± 0.87	29.03 ± 0.36	3.6	28.46 ± 0.48	1.6	28.98 ± 0.50	3.5	0.273	0.134
MCHC	g/L	33.15 ± 0.36	33.24 ± 0.44	0.3	33.36 ± 0.21	0.6	33.54 ± 0.30	1.2	0.377	0.089
RDW	%	13.36 ± 0.24	13.16 ± 0.17	-1.5	13.15 ± 0.17	-1.6	13.07 ± 0.22	-2.2	0.293	0.127
PLT	x 10^9/L	277.0 ± 23.01	192.4 ± 14.4 *	-30.5	270.9 ± 18.5	-2.2	252.5 ± 13.7	-8.8	< 0.001	0.558
MPV	fL	7.15 ± 0.33	6.85 ± 0.32	-4.2	7.28 ± 0.45	1.8	7.06 ± 0.48	-1.3	0.836	0.02
NEU	%	59.05 ± 3.84	59.54 ± 3.84	0.8	60.3 ± 2.65	2.1	57.43 ± 2.60	-2.7	0.560	0.069
MONO	%	6.16 ± 0.44	5.98 ± 0.38	-2.9	5.82 ± 0.42	-5.5	6.53 ± 0.40	6.0	0.243	0.146
EOSO	%	3.35 ± 0.65	3.15 ± 0.54	-6.0	3.65 ± 0.68	9.0	3.29 ± 0.72	-1.8	0.549	0.06
LYM	%	29.02 ± 3.08	29.12 ± 3.33	0.3	27.46 ± 2.18	-5.4	28.48 ± 2.82	-1.9	0.720	0.035

The percentage difference (Diff %) values shown refer to the difference between each variable at the given time compared to pre-donation. * - $p < 0.05$ compared to pre-donation. Abbreviations: Eta - partial Eta squared i.e. the percentage of variance that is accounted for by the effect of blood donation, Hb - hemoglobin, Hct - haematocrit, LYM - lymphocytes, MCV - mean cell volume, MCH - mean cell hemoglobin, MCHC - mean cell hemoglobin concentration, MPV - mean platelet volume, NEU - neutrophils, PLT - platelet count, RBC - red blood cells, RDW - red cell distribution width, Sig - the estimate of within-subject mean variation across different time-points using repeated ANOVA, WBC - white blood cells.

Table 2. Lymphocyte subsets by flow cytometry (mean ± SEM) in 10 healthy subjects pre- and (24 hours, 8 days, 22 days) post-donation.

	Unit	Pre-donation	24 hours	Diff %	8 days	Diff %	22 days	Diff %	Repeated ANOVA	
									Sig	Eta
CD3	%	68.3 ± 3.41	67.8 ± 3.58	-0.7	69.11 ± 3.28	1.2	68.7 ± 3.07	0.6	0.243	0.146
CD4	%	34.9 ± 1.83	34.7 ± 2.03	-0.6	35 ± 1.36	0.3	35.6 ± 1.57	2.0	0.615	0.061
CD8	%	27.7 ± 2.68	27.6 ± 2.60	-0.4	27.89 ± 2.76	0.7	27.3 ± 2.51	-1.4	0.821	0.018
CD19	%	12.7 ± 1.78	12.9 ± 1.9	1.6	13.33 ± 1.73	5.0	13.1 ± 2.07	3.1	0.712	0.041
CD16-56	%	18.4 ± 3.75	19 ± 3.95	3.3	16.67 ± 3.07	-9.4	17.9 ± 3.44	-2.7	0.617	0.056
CD4/CD8		1.38 ± 0.16	1.37 ± 0.16	-0.7	1.38 ± 0.17	0.0	1.44 ± 0.17	4.3	0.581	0.065

The percentage difference (Diff %) values shown refer to the difference between each variable at the given time compared to pre-donation. Abbreviations: Eta - partial Eta squared i.e., the percentage of variance that is accounted for by the effect of blood donation, Sig - the estimate of within-subject mean variation across different time-points using repeated ANOVA.

Hematological parameters

Data for CBC and WBC categories are presented in Table 1. RBC and Hct counts were lower than pre-donation values at visits B, C, and D following platelet donation. The progressive decrease in values ranged from -4.1% to -5.7% for RBC (Figure 2A). Values for MCV, MCH, and MCHC increased slightly post-donation but there were no significant changes ($p > 0.05$).

The total WBC increased 24 hours following platelet apheresis (6.3%) and returned to the baseline level at

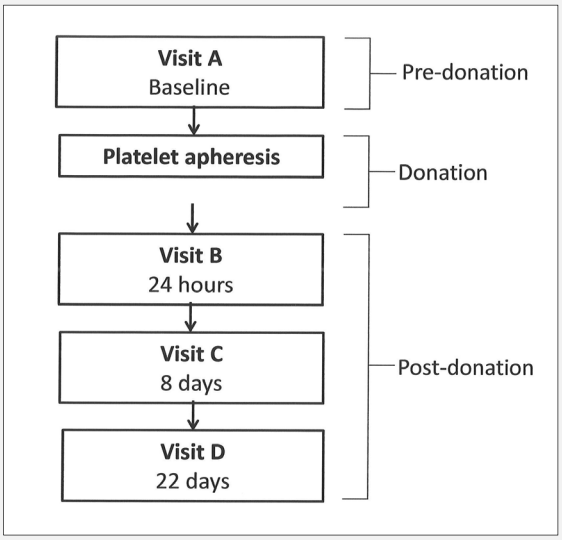

Figure 1. Protocol for the involved 10 subjects included in each visit and in between time interval.

visit B and decreased to -7.2% at visit C (Figure 2B). However, the differential WBC count showed that both neutrophil and lymphocyte fractions increased by 0.8% and 0.3%, respectively, at visit B.

None of the donors had a pre-donation platelet count of less than 200 x 10^9/L. The mean platelet count decreased significantly ($p < 0.05$) from the baseline 277.0 ± 23.0 x 10^9/L to 192.4 ± 14.4 x 10^9/L after 24 hours of platelet apheresis (Figure 2, C). The fall being almost 30% of the baseline value. However, by the time of visits C and D, the mean level of platelets appeared to be almost completely recovered, with a slight, non-significant, decrease of -2.2% and -8.8% compared to the baseline level ($p > 0.05$). In addition, none of the donors had a platelet count < 100 x 10^9/L post-procedure.

Lymphocyte subsets

Data on lymphocyte subsets measured by FC are presented in Table 2. At visit B after platelet donation CD3, CD4, and CD8 cells constituted a lower proportion of the total lymphocyte count compared to baseline (-0.7%, -0.6%, and -0.4%, respectively), but the decrease was non-significant. However, this decrease, together with the decreased proportion of CD4 and CD8 cells, resulted in a small, but non-significant reduction (-0.7%) in the CD4/CD8 ratio. The CD4/CD8 ratio returned to the baseline level at visit C, and increased at visit D (4.3%, $p > 0.05$). At 24 hours, the proportions of CD19 and CD16-56 (NK) as the percentage of total lymphocytes were slightly increased (1.6% and 3.3%, $p > 0.05$ respectively). The proportions of both CD19 and NK cells were not significantly increased throughout the study period.

DISCUSSION

In this study, we followed changes in CBC and lymphocyte subsets over 22 days, a longer period than in previous studies [3,6,7]. In line with the findings of previous studies, Lewis et al. had no spontaneous changes in Hb, RBC, and Hct levels after platelet apheresis [7]. Blood loss within the harness (Gambro BCT) and at sampling on the Trima Accel cell separator is between 80 and 100 mL. This is normally followed by an immediate redistribution of fluid from the extravascular to intravascular space which restores the lost blood volume, which could explain the slightly reduction in levels of Hb, RBC, and Hct. Hemodilution caused by infusions of citrate solutions and saline could be another reason for the reduction in hematological values after platelet apheresis. The latter has been reported previously in other studies [3,8]. Whilst these hematological changes have previously been described elsewhere [9-11], it was reassuring to confirm that the responses were as expected. In our previous study of whole blood donation, the decrease in platelet count 24 hours post donation was only 0.8% [12]. Therefore, the noted decrease in platelet count in this study at 24 hours post-platelet apheresis can be explained by the process of pheresis itself where the removal of about 30% of the platelets from the whole blood is the desired outcome.

Reference change value (RCV) is defined as the critically significant changes between two results. It is important for determining whether apparent changes in serial results reflect critical changes or can be accounted for by biological variation alone. Data on biological variations are essential for calculating the reference change

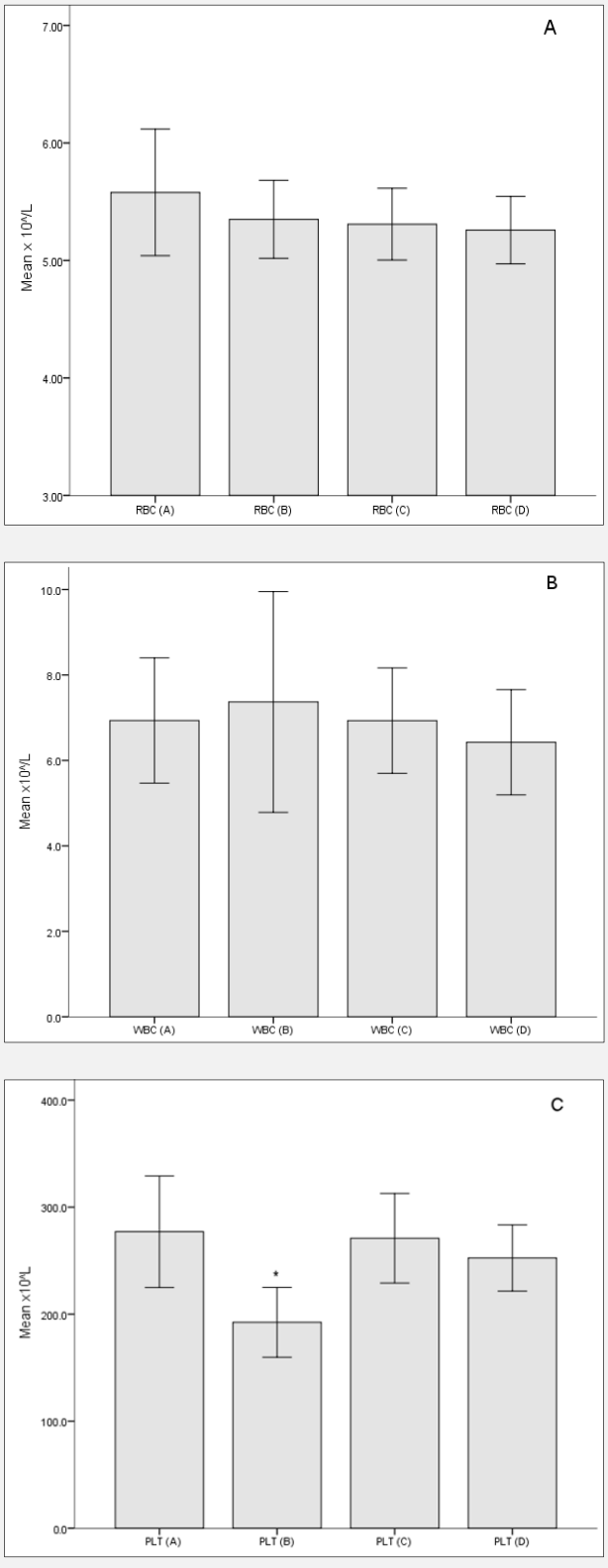

Figure 2. Changes in the means of, RBC (Figure A), WBC (Figure B), and PLT (Figure C) before (visit A) and after (visits B, C, and D) platelet apheresis.

*** - $p < 0.05$, when compared to the baseline level (visit A). Bars represent 95% CI.**

value (RCV), which is especially useful for defining critical laboratory results [13]. The RCVs for the platelet count were calculated earlier by Buoro et al., using the same Sysmex analyzer and it was found to be 20.2% and 9.4% for the medium (one week) and short-term (one day) intervals, respectively [13]. Both RCVs are less than the noted 30.5% change in PLT counts in our subjects after 24 hours at visit B, which means that PLT count has critically changed and should be considered for attention before any further donation on the same day. However, changes in PLT counts at visit C (-2.2%) and D (-8.8%) were less than medium and short-term values for RCV. Similar findings were reported by Wagner et al., where platelet count returned to the base line level by day 7 post-platelet apheresis [14]. Furthermore, in the same study the thrombopoietin (TPO) level was found to increase from day 1 to day 7, and colony forming unit-megakaryocytes (CFU-Mk) continuously increased from day 1 to day 4 also and showed a decrease by day 7 [14]. The increase in serum TPO and CFU-Mk have been reported to provide evidence for the recovery trend seen in platelet count [14]. Therefore, the time of platelet recovery and trend which were observed by Wagner et al. are consistent with our results. So, it can be concluded that it will be safe to donate PLT after one week of platelet apheresis but not after 24 hours. In this study a slight and unexpected increase in total WBC count (6.3%) at 24 hours after PLT donation was noted. Reduction in WBC post-platelet apheresis had been observed in previous studies [3,4] which were designed to estimate WBC count immediately after platelet apheresis while in our study the estimation of WBC count was after 24 hours. The increase in neutrophils (0.8%) after visit B of platelet apheresis in our study may reflect the recovery time point of sampling following the acute insult of PLT donation. Possible causes of neutrophil leukocytosis include infection or inflammation but these are unlikely in this study of healthy donors. A more likely reason is increased secretion of the stress hormones cortisol and epinephrine [15]. These hormones are secreted in response to blood loss resulting in mobilization of leukocytes from bone marrow and from the marginalized pool of neutrophils adherent to blood vessel walls.

PLT donation had no significant effect on the total lymphocyte count, nor upon the lymphocyte subsets. Similarly, other investigators have shown no changes in lymphocyte numbers in donors after frequent apheresis [16,17], or have documented transient changes returning to post-donation levels over the duration of repeated platelet apheresis [18].

We observed a slight, but non-significant, change in mean CD16-56 (NK) at eight days (-9.4%). A significant decrease has been observed in patients who have undergone blood donation in other studies [19,20], with 54% of the CD16-56 (NK) cells pre-donation levels remaining at 12 days post-donation. This suggests that regular platelet apheresis may result in a reduction in NK cell count.

There was no significant change in CD8, CD3, and CD4 cells, but they were slightly increased after eight days with no change in the CD4/CD8 ratio. Various lines of evidence suggest that a declining CD4/CD8 ratio correlates with immune dysfunction leading to a poor response to immunization, as well as an increased risk of severe infections and malignancies [21,22]. The decrease also predicts mortality in patients with viral infection and in the general population [23]. Therefore, the non-significant change in CD4/CD8 ratio in response to PLT donation may indicate a steady state of immune response. In contrast, Matsui et al. [24] reported that total T cells, CD4 cells, CD8 cells, CD57 lymphocytes, and CD4/CD8 ratio were all decreased, but they found an increase in B cells and monocytes. However, the platelet apheresis procedure used in their study causes a greater loss of lymphocytes than apheresis equipment currently in use. This is in contrast to the findings in our previous study where patients had undergone whole blood donation [12]. In the whole blood donation study, we demonstrated a significant increase in CD4/CD8 ratio seen at Day 22 and in CD19+ cells at day 8. Erythropoietin levels were unsurprisingly increased especially at day 8. Whether or not the changes in lymphocyte subsets observed are related to the elevation in erythropoietin (EPO) level has yet to be clarified. In this study, we assume that platelet apheresis would not have any significant effect on EPO levels, as the process of donation does not involve the removal of significant number of red cells. Therefore, a reduction in oxygen tension to trigger the release of erythropoietin is not expected. Therefore, we think that the changes observed in this study, apart from platelet counts, were not clinically significant.

This study has a number of limitations, principally the relatively small number of subjects studied throughout. However, most previous studies were conducted on similarly small numbers. This is due to the nature of the repeated measures.

CONCLUSION

In conclusion, after 7 days, a donor's PLT count returned to baseline. The significant reduction in PLT count at 24 hours after platelet apheresis may have adverse health effects on PLT donors. Changes in lymphocyte subsets reveal that the process of platelet apheresis has no immunomodulatory effect on the immune system.

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Ethical Approval:
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Declaration of Interest:
None competing interests.

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