

Influence of Regular Plateletpheresis Donations on Immature Platelet Fraction and Platelet Count

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Received: 23 December 2024 / Accepted: 19 May 2025

ABSTRAK

Pendermaan plateletferesis boleh dilakukan dengan lebih kerap berbanding pendermaan darah penuh. Kajian ini bertujuan untuk menerokai hubungan antara kekerapan, selang masa antara pendermaan dan kesan pendermaan plateletferesis jangka panjang terhadap kiraan platelet dan fraksi platelet tidak matang (IPF). Hasil daripada kajian ini diharapkan dapat memperkukuhkan data keselamatan bagi pendermaan plateletferesis. Seramai 244 orang penderma telah dikaji dan dibahagikan mengikut jenis pendermaan, iaitu penderma plateletpheresis ($n = 184$) atau penderma darah penuh ($n = 60$). Sampel darah peserta dianalisis menggunakan Sysmex XE-5000 untuk mendapatkan kiraan darah penuh dan IPF. Kiraan platelet asas diperolehi daripada rekod sebelum pendermaan pertama dan dibandingkan dengan kiraan platelet sebelum pendermaan terkini bagi semua penderma plateletferesis. Hubungan antara kekerapan, selang masa dan tempoh pendermaan plateletferesis dengan kiraan platelet dan IPF turut dinilai. Penurunan signifikan secara statistik bagi kiraan platelet ($273 \times 10^9/L$, 172-443) diperhatikan apabila dibandingkan dengan kiraan asas sebelum pendermaan ($291 \times 10^9/L$, 164-478) dalam kalangan penderma plateletferesis. Walau bagaimanapun, penurunan ini tidak membawa kesan klinikal kerana kiraan platelet masih dalam julat normal. Pemerhatian yang sama juga dilihat tanpa mengira selang masa atau tempoh pendermaan. Nilai IPF dalam kalangan penderma plateletferesis (1.5%, 0.5-7.5) juga setanding dengan penderma darah penuh (1.5%, 0.6-4.8; $p=0.848$). Pendermaan plateletferesis secara kerap dengan selang masa sekurang-kurangnya 14 hari antara pendermaan, serta jumlah pendermaan sehingga 24 kali setahun tidak menyebabkan trombositopenia atau peningkatan IPF. Penemuan ini diharapkan dapat menggalakkan lebih ramai penderma menyumbang, seterusnya membantu perkhidmatan transfusi darah memenuhi permintaan yang semakin meningkat terhadap produk plateletferesis.

Kata kunci: Fraksi platelet tidak matang; plateletferesis; pendermaan darah

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ABSTRACT

Plateletpheresis donation could be done more frequently than whole blood donation. This study is designed to explore the correlation between frequency, inter-donation intervals and effects of long-term plateletpheresis donation with the platelet count and immature platelet fraction (IPF). Outcomes from this study will strengthen the safety data for plateletpheresis donation. A total of 244 donors were studied. They were grouped into plateletpheresis (n=184) or whole blood donors (n=60). Participants blood samples were collected and analysed for full blood count and IPF by Sysmex XE-5000. Baseline platelet counts were retrieved from records before the first donation and compared to the most recent pre-donation counts for all the plateletpheresis donors. Finally, the influence of frequency, interval and total duration of plateletpheresis donation on the platelet count and IPF were assessed. Statistically significant reduction of platelet count ($273 \times 10^9/L$, 172-443) were observed when compared with the baseline pre-donation count ($291 \times 10^9/L$, 164-478) for plateletpheresis donors. However, this fall is not of clinical significance as platelet counts were still within normal ranges. Similar observation was made regardless of inter-donation interval and total duration of donation. IPF for the plateletpheresis donors (1.5%, 0.5-7.5) were also comparable with the whole blood donors (1.5%, 0.6-4.8; $p=0.848$). Frequent plateletpheresis donation with at least 14 days inter-donation intervals and up to 24 donations a year does not cause thrombocytopenia or increase in the IPF. This knowledge is hoped to encourage more people to become donors thus helping blood transfusion services to meet the increasing demand for plateletpheresis product.

Keywords: Blood donation; immature platelet fraction; plateletpheresis

INTRODUCTION

The automated apheresis technique was first developed in the 1970s (McLeod 2010) and it has since become one of the main services provided by the Malaysian National Blood Centre and other transfusion services.

With the increasing demand of blood products, the transfusion services could not rely only on platelet concentrate produced from the whole blood donation. This is because to get therapeutic dosage for an adult patient from the platelet transfusion, at least four units of platelets are required which would expose the patient to four different donors if platelet concentrates from whole-blood donors were used. Short shelf life of platelet concentrates of five days and long whole blood donation interval of two months (National Blood Centre 2008) are the other factors that will limit the availability of platelet concentrate.

According to data from the WHO Global Database on Blood Safety (World Health Organisation 2008), the voluntary non-remunerated blood donor for developing countries is around 3.6 donations per 1000

population which is far less compared with 45.4 donations per 1000 population in the developed countries making the inventory management even more challenging in Malaysia.

Since the introduction of the automated apheresis platelet donation, donors can now donate platelet every two weeks and up to 24 times a year and the therapeutic dose for platelet transfusion can be easily achieved from a single donor. Besides reducing the number of donor exposure, the plateletpheresis donor can be tailored to the clinical need of HLA-matched platelet or IgA-deficient platelet and the collected platelets are leucoreduced.

The known adverse effects of apheresis donation are hemolysis, air embolism, significant hematoma, allergic reactions and citrate toxicity (Heuft et al. 2013). However, in the past years, a possible decrease in platelet count in long-term plateletpheresis donors has been analysed by several authors with contradictory results. Stohlawetz et al. (1998) found no significant difference between baseline platelet count in a group of first-time apheresis donors and in

another group, who had donated approximately every two weeks for more than 18 months. Katz et al. (2007) mirrored these findings.

In contrast, Nadiyah et al. (2013) noted that there was a statistically significant reduction of the post plateletpheresis when compared with pre plateletpheresis platelet count, although the reduction was not clinically significant.

Immature platelet fraction (IPF) is the percentage of immature platelet compared to the total platelet count. It is elevated when there is increase in the production of new platelet in response to thrombocytopenia for example in the case of idiopathic thrombocytopenia purpura (Abe et al. 2006). IPF is used to predict engraftment in post-transplant patient and as an early indicator of marrow recovery post chemotherapy (Briggs et al. 2006; Yamaoka et al. 2010). Therefore, in regular plateletpheresis donor, the IPF would possibly be raised if the reduction of the platelet count due to donations is clinically significant.

This study aimed to determine the correlation between frequency, interval between donations and effects of long-term platelet apheresis donation with the platelet count and immature platelet fraction. Findings will further provide safety profile for long-term plateletpheresis donation.

MATERIALS AND METHODS

This was a cross-sectional study on plateletpheresis donor in National Blood Center, Kuala Lumpur with retrospective data retrieval from plateletpheresis donor record. This study was conducted over a period of nine months from January 2014 until September 2014. The subject group comprised of plateletpheresis donor and the control group comprised of whole blood donor. There were a total number of 244 people enrolled into this study; comprised of 184 subjects and 60 controls.

The demographic data, baseline full blood count (FBC) result and data regarding plateletpheresis duration, frequency and interval were recorded. 3 mL of whole blood was

obtained prior to apheresis procedure and tested for FBC and reticulocytes count in the Pathology Department, General Hospital Kuala Lumpur as soon as possible or within eight hours after sample collection. The platelet count from the donors obtained during the donation day were compared with the baseline platelet count which was defined as the platelet count prior to the first plateletpheresis donation as obtained from the donor record. The platelet count and IPF were also compared with the control group.

FBC and IPF measurements were performed for all the study samples using Sysmex XE-5000 (Sysmex Corp., Kobe, Japan). Parameters measured include haemoglobin (Hb), platelet count and IPF. The Sysmex XE-5000 incorporated fluorescence flowcytometry into a multichannel instrument that also utilised laser light and direct current (for impedance measurements) to perform a differential count and optical platelet count. For IPF measurement, two fluorescent dyes, polymethine and oxazine entered the cells and binded to their DNA and RNA. The stained cells were then analysed as they passed through a semiconductor diode laser beam, where both forward scatter light (representing cell volume) and fluorescence intensity (indicating RNA content) were detected. Mature and immature platelets were distinguished based on their fluorescence intensity and the IPF was reported as a proportional value (IPF %) relative to the total platelet count using the optical method.

Meanwhile, plateletpheresis procedure was performed using various apheresis machines according to the donors' preference. Machines in use include AMICUS (Baxter Fenwal Division, Deerfield, IL), MCS+ (Haemonetic Corp, Braintree, MA), Spectra LRS (COBE BCT, Lakewood, CO) and Trima (Gambro BCT, Lakewood, CO). Acid-citrate- dextrose formula A (ACD-A) was used as anticoagulant during procedures according to the manufacture's recommendations.

Data obtained from all participants were recorded and analysed using SPSS version 12 (IBM Corp. Armonk, NY, USA). Non-parametric tests were applied, and a p-value < 0.05 was considered to be statistically significant.

RESULTS

A total of 244 individuals (219 males and 25 females) were enrolled into this study for nine months duration from January 2014 until September 2014. The sample populations according to the races were 127 (52%) Malay, 91 (37%) Chinese, 22 (9%) Indian and 4 (2%) from other races. All of the sample populations were healthy individuals aged between 17 to 62 years old.

The individuals involved in this study were divided into two main categories; plateletpheresis donor (184 individuals) and whole blood donor were used as control group (60 individuals).

The latest plateletpheresis donors' predonation red blood cells parameters from the sample obtained during the study recruitment showed no marked difference when compared with the baseline predonation red blood cells parameters (Table 1).

Interestingly, there was a reduction of platelet count observed in the plateletpheresis donors when the latest platelet count was compared with

the baseline platelet count, $p < 0.001$. However, the reduction of platelet count was not clinically significant (median platelet count was $291 \times 10^9/L$ versus $273 \times 10^9/L$) (Table 2).

Despite the above finding, there was no statistically significant difference in the latest predonation platelet count observed in plateletpheresis donors when they were categorised according to the frequency, intervals and duration of plateletpheresis donation, $p > 0.05$ (Table 3, 4 & 5). Analyses were done with Kruskal-Wallis test with sample size of 183.

Immature platelet fractions observed in the whole blood donors were comparable with the plateletpheresis donors. Figure 1 showed boxplot comparing IPF level between whole blood donors and plateletpheresis donors. Both groups have similar median and comparable interquartile ranges. There were five plateletpheresis donors recorded IPF of more than 5% but none in the whole blood donors group (Table 6). However, the differences were not statistically significant, $p = 0.85$ (Table 7).

TABLE 1: Mean, SD and range of Hb, MCV and MCH for the plateletpheresis donor at 2 different settings

Parameters	Baseline predonation parameters for plateletpheresis donors*		Latest predonation parameters for plateletpheresis donors#	
	Mean + SD	Range	Mean + SD	Range
Hb, g/dL	14.93 + 1.04	12.00-17.40	14.80 + 1.02	12.40-17.40
MCV, fL	86.22 + 6.07	62.90-103.00	84.42 + 6.07	61.40-97.80
MCH, pg	28.92 + 2.48	19.30-34.90	28.46 + 2.41	19.80-33.50

* Baseline predonation parameters were obtained from the donors' record.

Latest predonation parameters obtained during the research recruitment phase.

SD: Standard deviation; Hb: Hemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin

TABLE 2: Baseline predonation platelet count compared with the latest predonation platelet count

Parameters	Baseline platelet count for plateletpheresis donor		Latest platelet count for plateletpheresis donor		P value*
	Median (IQR)	Range	Median (IQR)	Range	
Platelet count $\times 10^9/L$	291 (78)	164-478	273 (57)	172-443	<0.001

*Analysed with Wilcoxon Signed Rank test, $n = 183$

IQR: Interquartile range

TABLE 3: Predonation platelet count according to the interval of the plateletpheresis donation

Parameters	13-20 days interval (n = 74)		>20 days interval (n = 109)		p-value*
	Median (IQR)	Range	Median (IQR)	Range	
Platelet count x10 ⁹ /L	274.5 (44)	204-478	271 (60)	164-463	0.946
IQR: Interquartile range					

TABLE 4: Predonation platelet count according to the duration of plateletpheresis donation

Parameters	<1 years (n = 30)		1-2 years (n = 23)		>2 years (n = 130)		p-value*
	Median (IQR)	Range	Median (IQR)	Range	Median (IQR)	Range	
Platelet count x10 ⁹ /L	276 (46)	164-382	265 (65)	199-414	274.5 (57)	199-478	0.268
IQR: Interquartile range							

TABLE 5: Predonation platelet count according to the frequency of plateletpheresis donation in 2 years

Parameters	Control (n = 60)		1-20 times (n = 95)		21-40 times (n = 67)		>40 times (n = 21)		p-value*
	Median (IQR)	Range	Median (IQR)	Range	Median (IQR)	Range	Median (IQR)	Range	
Platelet count x10 ⁹ /L	281.5 (90)	147-437	275 (60)	164-383	275 (48)	199-463	272 (37)	199-478	0.964
IQR: Interquartile range									

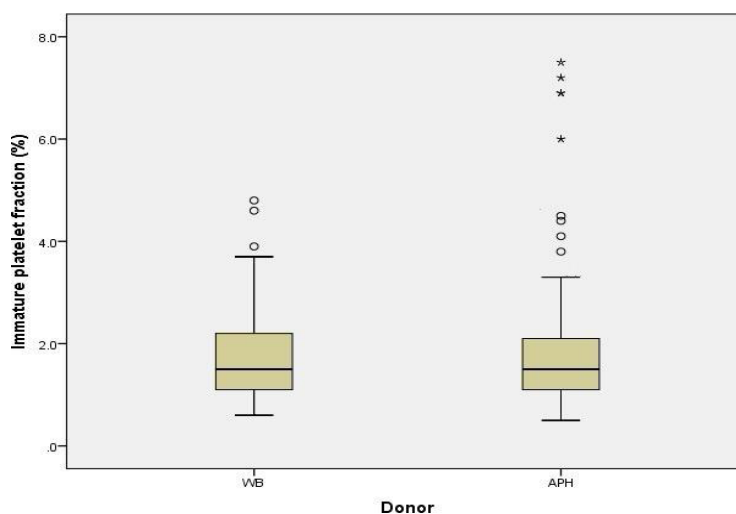


FIGURE 1: Boxplot for predonation IPF levels according to whole blood and plateletpheresis donors. Asterisks and circles represented outliers with IPF value more or less than 5% respectively. Donors marked with asterisks were further described in Table 6. (IPF: Immature platelet fraction; WB: Whole blood; APH: Apheresis)

TABLE 6: Characteristics of 5 plateletpheresis donors with predonation IPF values of more than 5%

Donor	Baseline platelet count, x10 ⁹ /L	Recent platelet count, x10 ⁹ /L	IPF, %	Interval platelet-pheresis donation, days	Duration of platelet-pheresis donation, days	Frequency of platelet-pheresis donations, numbers*
A	172	196	6.0	129	180	3
B	201	201	6.9	92	609	9
C	221	211	6.9	14	3923	27
D	233	209	7.2	21	1890	15
E	236	278	7.5	112	707	22

*Number of plateletpheresis donations in 2 years
IPF: Immature platelet fraction

TABLE 7: Predonation IPF values in whole blood and plateletpheresis donors

Parameters	Whole blood donors (n=58)		Plateletpheresis donors (n=157)		p-value*
	Median (IQR)	Range	Median (IQR)	Range	
IPF, %	1.5 (1.1)	0.6-4.8	1.5 (1)	0.5-7.5	0.848

*Analysed with independent sample Mann-Whitney U test
IQR: Interquartile range; IPF: Immature platelet fraction

There was no strong correlation between the interval, duration and frequency of plateletpheresis donation with the raised IPF level in the five plateletpheresis donors (Table 6). None of the plateletpheresis donors have thrombocytopenia, only 1 donor had minimum 14 days interval and no donor had more than 40 times plateletpheresis donation in 2 years' time.

DISCUSSION

The plateletpheresis donations are relatively safe. However, some studies have postulated that regular plateletpheresis donation may lead to reduction in platelet count and this may be due to clinical problem.

Long term and frequent plateletpheresis donation possibly stimulate haemopoietic systems. According to Drummond et al. (2007), telomere length can offer insights on the replicative history of the cells in question. The telomere length will be shortened in highly

proliferating cells (Drummond et al. 2004). However, study done by Scheduling et al. (2003) showed there was no difference in the telomere length between the donors and non-donors. As indicated by telomere length analysis, the impact of frequent blood donations on the replicative capacity of the human haemopoietic stem cells pool seems to be negligible.

Platelet production is regulated by thrombopoietin, which is modulated by platelet count. Low platelet count results in increased thrombopoietin level which then will stimulate megakaryopoiesis and platelet production (Mc Carty et al. 1995). Reticulated platelet is a new and young platelet which rich in RNA content and bigger in size. Therefore, it could be measured by flowcytometry and determined by bigger forward scatter and higher fluorescence intensity relative to total number of platelet and reported as immature platelet fraction. IPF typically increased in disorders of platelet destruction (Abe et al. 2006) and it could

possibly increase in plateletpheresis donors who had thrombocytopenia.

This study noted a statistically significant drop in the latest predonation platelet count compared with the baseline platelet count ($291 \times 10^9/L$ (164-478) vs. $273 \times 10^9/L$ (172-443), $p < 0.001$). However, the reduction of platelet count was not clinically significant as no donor had platelet count lower than $150 \times 10^9/L$.

Our findings also suggested that the intervals, frequency and duration of plateletpheresis donation were not related with the reduction of the platelets count. A number of studies have demonstrated that multiple plateletphereses do not result in significant differences in the plateletpheresis donor platelet count (Katz et al. 2007; Richa et al. 2008; Stohlawetz et al. 1998). There are no definitive reports to support that more than 24 plateletpheresis donation per year is harmful to the donors.

The reduction of platelet count observed in this study also were not clinically significant with lowest platelet count, $172 \times 10^9/L$. The reduction of platelet counts also did not increase the IPF in the plateletpheresis versus whole blood donors, suggesting that the platelet reductions observed in this study were not significantly enough to increase the production of new platelets. This confirms the findings of Scheduling et al. (2003) that long-term whole blood and platelet donation does not significantly increase stem cell turnover.

This is in line with Food and Drug Administration (2007) guidance that suggested a donor should undergo no more than 24 plateletpheresis collections in 12 months period to ensure the safety of the donor. Most of the plateletpheresis donors donated apheresis platelet less than 24 times per annum with a minimum interval of 14 days.

CONCLUSION

In conclusion, the plateletpheresis donation is a safe procedure and more whole blood donor could be encouraged to enter the apheresis program and the existing plateletpheresis donors could safely donate more frequently to ensure

continuous platelets supply for clinical use.

Author contributions: Study design: RZARS; Data collection, laboratory work: ANM; Data analysis: LHMP; Writing-original draft: LHMP; ANM; Writing-review and editing: QAH; Supervision: AH, NM, RO, WNH, RZARS; Funding: RZARS. All authors have approved the final manuscript.

Conflict of interest: The authors declare no conflicts of interest.

Funding: This research was supported by Fundamental Grant of Faculty of Medicine, Universiti Kebangsaan Malaysia (FF-217-2013).

Acknowledgement: The authors would like to express their gratitude to Madam Habitah Ishak, Miss Noor Razleen binti Zaharuddin, Mr Mohamad Adam Hj. Bujang and all nurses of National Blood Centre.

Ethics statement: This study was approved by the Research Ethics Committee of Universiti Kebangsaan Malaysia (FF-217-2013), National Institutes of Health (Clinical Research Centre, CRC) and National Medical Research Register (NMRR) of Ministry of Health Malaysia (NMRR-12-1367-14514). Informed consents were obtained from platelet apheresis donors enrolled as well as from the whole blood donors.

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