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Original Article

Haemostatic parameters, platelet activation markers, and platelet indices among regular plateletpheresis donors

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المخلص

أهداف البحث: يعتبر فصل صفائح الدم لإعدادها للتبرع إجراءً آمناً، بصورة عامة. وهناك دراسات محدودة عن تأثيرها على معلمات تخثر الدم، واحتمال وجود ارتباط بين الفصل الآلي للصفائح الدموية وحالة فرط التخثر. من أجل ذلك أجريت هذه الدراسة للتحقيق في تأثير فصل صفائح الدم على المتبرعين المنتظمين باستخدام معلمات تخثر الدم التالية؛ البروتينات الطبيعية المضادة للتخثر، ومؤشرات الصفائح الدموية ومؤشرات تنشيط الصفائح الدموية.

طرق البحث: تم توظيف 139 شخصاً (ضوابط سليمة ومتبرعين بالصفائح الدموية عن طريق الفصل الآلي للصفائح الدموية)، كما تم تقسيمهم إلى مجموعتين. أجريت اختبارات مؤشرات التخثر والصفائح على أفراد المجموعة الأولى، في حين أجريت اختبارات مؤشرات تنشيط الصفائح الدموية باستخدام سي دي 62 بي والأجسام المضادة أحادية النسيلة باك-1 على أفراد المجموعة الثانية.

النتائج: تبين وجود تقلص بسيط ذي قيمة في مدة البروثرومبين وتنشيط الصفائح الدموية (وذلك بزيادة معلمات سي دي 62 بي والأجسام المضادة أحادية النسيلة باك-1) بين المتبرعين المنتظمين بالصفائح عن طريق الفصل الآلي للصفائح الدموية بالمقارنة بالمتبرعين من الضوابط السليمة. ولوحظ انخفاض كبير في عدد الصفائح الدموية قبل التبرع الحالي بين المتبرعين المنتظمين بالصفائح عن طريق الفصل الآلي للصفائح الدموية مقارنة بمتوسط تعداد صفائحهم الدموية قبل أول تبرع لهم بالصفائح عن طريق الفصل الآلي للصفائح الدموية. إلا أنه لم يُلاحظ فرق ذو قيمة لمؤشرات الصفائح الأخرى (عدد الصفائح والحجم المتوسط للصفحة و عرض توزيع الصفائح ومدة الثرومبولاستين الجزئي المنشط

وبروتين سي وبروتين إس ومضاد الثرومبين ومولد المضاد لعامل فان وليبراند) عند المقارنة بالمتبرعين من الضوابط السليمة.

الاستنتاجات: تخلص هذه الدراسة إلى أن الفصل الآلي للصفائح الدموية بصورة منتظمة هو إجراء آمن. ويتطلب احتمال التنشيط البسيط للصفائح الذي قد يحدث لدى المتبرعين المنتظمين، مزيداً من التأكيد. إلا أنه قد يحدث تنشيط للصفائح وعامل التخثر السابع، في المختبر، قبل التحليل، الأمر الذي قد يسهم في حدوث هذه الملاحظات.

الكلمات المفتاحية: معلمات تخثر الدم؛ الفصل الآلي للصفائح الدموية؛ مؤشرات الصفائح الدموية؛ مؤشرات تنشيط الصفائح الدموية

Abstract

Objective: Plateletpheresis is generally a safe procedure for platelet donation. Studies on the effects of haemostatic parameters and possible association between automated plateletpheresis and hypercoagulable state are limited. Hence, this study aimed to investigate the effects of plateletpheresis on regular donors using haemostatic parameters, i.e. natural anticoagulant proteins, platelet indices, and platelet activation markers.

Methods: A total of 139 participants (plateletpheresis donors and normal controls) were recruited and divided into two groups: Group 1 participants who underwent tests for haemostatic and platelet indices and Group 2 participants who underwent tests for platelet activation markers using CD62P and PAC-1 monoclonal antibodies.

Results: A significant mild shortening of prothrombin time and platelet activation were demonstrated (by increased CD62P and PAC-1 markers) among regular plateletpheresis donors as compared to healthy controls. The current pre-donation platelet count of plateletpheresis

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donors was significantly lower than their mean baseline platelet count obtained before their first plateletpheresis procedure. However, no significant differences were observed for the other platelet parameters (platelet count, mean platelet volume, platelet distribution width, activated partial thromboplastin time, protein C, protein S, antithrombin, and von Willebrand Factor antigen) between plateletpheresis donors and healthy controls.

Conclusion: This study concludes that regular plateletpheresis is a safe procedure. A possibility of mild platelet activation among regular donors requires further confirmation. However, pre-analytical platelet and FVII activations could occur in vitro contributing to these findings.

Keywords: Haemostatic parameters; Plateletpheresis; Platelet indices; Platelet activation markers

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Introduction

Possible changes in haemostatic system and platelet function among regular plateletpheresis donors have been reported. Hypercoagulable state is one of the important issues that require further exploration, which potentially complicates regular plateletpheresis donors. Apheresis donation is routinely practiced by most blood centres worldwide. Since blood donation is usually non-remunerated, its safety issues should be discussed and ascertained. Whole blood and apheresis donations are generally safe¹ because immediate complications are usually mild, which do not require medical attention. Serious and clinically significant complications are unusual. However, studies assessing the effects of repeated donations especially on the haemostatic aspects of plateletpheresis are limited.

Apheresis donations are more frequently performed compared to whole blood donations. In general, an apheresis donor must meet the requirements of a whole blood donor established by the blood centre. More stringent assessment is required for donors who participate in a serial apheresis program.²

Apheresis technology, even though known as safe and efficient, carries some additional risks to the donors. The apheresis technique shares the same reactions and injuries observed with whole blood donations and also has unique complications due to the frequency and methods of collection.¹ Overall, lesser reactions are observed with apheresis donation than with whole blood donation; however, the risk of more severe reactions, requiring hospitalisation, is higher with the former.³ A study on the frequency of immediate adverse effects associated with apheresis donation showed a similar finding that the most common complications are venipuncture-related complications (haematoma, pain).^{3,4}

Plateletpheresis procedure leads to immediate loss of large proportion of circulating platelets⁵ as well as sustained

decreases in platelet count after long-term plateletpheresis donations⁶; immediate platelet reduction has been proven by many studies. Hence, The American Association of Blood Banks recommended a pre-collection platelet count to enhance donor safety.^{3,33}

Platelet activation has been implicated in the pathogenesis of several diseases, such as atherosclerosis, coronary vascular disease, and cerebrovascular disease. It comprises a change in the platelet shape, platelet aggregation, and release of platelet constituents.

The experience from the widespread practice has enabled the development of a possible safe plateletpheresis procedure, especially with the newer generation machines.⁵ However, most studies only addressed the problems on immediate effects following plateletpheresis, and studies on platelet activation and hypercoagulable markers are limited. Therefore, careful donor selection and assessment of platelet activity should be conducted to eliminate possible complications that may affect a small group of high-risk donors.

A recent report suggested that apheresis procedure may induce transient hypercoagulable state based on some immediate abnormalities in the coagulation markers detected post-procedure. A case of a donor with inherited thrombophilia (heterozygous for methylene tetrahydrofolate reductase (MTHFR) 677C-T mutation and prothrombin 20210G-A allele) who had fatal cerebral infarction following a large volume plasma donation was reported.⁷ This case suggests that large volume plasma donation may trigger arterial thrombotic events in a donor with inherited thrombophilia. Fortunately, no fatal thrombotic complication has been reported in regular plateletpheresis donation. Probably some complications are under-reported as donors may present, in later time, with episodes of transient ischaemic attack, stroke, or acute coronary syndrome without investigating their connection with platelet reactivity.

Studies on the effects of plateletpheresis among regular donors were also extremely limited. Based on the literature review, we could not find current articles related to this study. Studies on the effects of plateletpheresis on platelet indices and blood coagulation parameters among regular plateletpheresis donors were found.^{8,9} However, these studies addressed coagulation parameters immediately post plateletpheresis donation. Therefore, no study has addressed the baseline coagulation status, especially the anticoagulant proteins, among regular plateletpheresis donors. Therefore, whether this procedure can influence the baseline haemostatic platelet indices and haemostatic markers following regular donations remains to be explored.

This study aimed to investigate the effects of plateletpheresis procedure on specific haemostatic parameters for hypercoagulability and platelet activation markers by comparing the results with healthy non-plateletpheresis controls. The regular committed plateletpheresis donors should be the best candidates and the sample should not be taken immediately from post-donation blood, because prothrombotic haemostatic markers are expected to be present during the procedure due to extracorporeal activation. In addition, baseline haemostatic parameters would be the best biomarkers for sustained hypercoagulable state, collected at the time interval prior to the next platelet donation procedure. This may predict the changes of haemostatic markers in

the regular plateletpheresis donors, especially the risk of developing vascular events.

Expected benefits from this study would be on the monitoring aspects of plateletpheresis donors and ensuring safe haemostatic function. In addition, proper actions could be addressed during the medical assessment of regular plateletpheresis donors.

Materials and Methods

Reference population and study locations

A prospective cross-sectional study was conducted in Hospital Pulau Pinang and Hospital Universiti Sains Malaysia (HUSM). A total of 139 participants (61 regular plateletpheresis donors and 78 healthy controls) were recruited and divided into two groups: Group 1, participants (41 plateletpheresis donors and 58 healthy controls) who underwent tests for haemostatic markers and platelet indices; and Group 2, participants (20 plateletpheresis donors and 20 healthy controls) who underwent tests for platelet activation markers using CD62P and PAC-1 monoclonal antibodies by flow cytometry analysis. Regular plateletpheresis is defined as consecutive plateletpheresis donation of at least ≥ 5 times within the last 18 months prior to the sample collection.⁶ Verbal and written consents were obtained prior to the blood collection. The age range for both participants and controls were between 18 and 60 years old. This study was approved by the institutional ethical committee of both hospitals, which complies with the declaration of Helsinki.

Blood collection and preparation

Blood was collected into two citrated containers (2.7 mL each) and one ethylenediaminetetraacetic acid container (3 mL). These samples were used for evaluating complete blood count, prothrombin time (PT), activated partial thromboplastin time (APTT), protein C, protein S, and antithrombin (AT) activities including von Willebrand Factor (vWF) antigen level.

For the platelet activation markers, special care was taken to minimise platelet activation during blood collection. The first 1 mL of blood was discarded and the subsequent 2.7 mL was collected into a 3.2% trisodium citrate container. The sample was then stained within 10 min to analyse CD62P and PAC-1 using a flow cytometer. All blood specimen collection, storage, and transport followed the Clinical Laboratory Standards Institute (H21-A5) guideline for haemostatic tests.

Haemostatic tests

In this study, haemostatic tests included protein C, protein S, and AT activity assays such as immune-turbidimetric assays of vWF antigen and routine PT and APTT tests. All tests were conducted using the Compact STAGO Coagulation Analyzer STA[®] based on its manual instructions (Diagnostica Stago, Inc., Asnières, France). Prior to the analysis, frozen PPP was thawed in a 37 °C water bath for 10 min before being transferred into bullet tubes. The results were represented as percentage for the assays and seconds for PT and APTT.

Protein C is activated in the presence of specific activators extracted from *Agkistrodon contortrix* venom. The activated protein C inhibits factors V and VIII and thus prolongs the APTT of the sample being tested. The normal range of protein C in the adult population is usually between 70% and 130%.¹⁰

The principle of protein S activity was based on the cofactor activity of protein S on the anticoagulant action of activated protein C. The normal range used during this study was between 55% and 140% (established in the local laboratory using normal volunteers).

AT provides an immediate and powerful inhibitory action on thrombin when heparin is present. The quantitation was based on the amyolytic action of the synthetic chromogenic substrate CBS 61.50. The normal plasma AT level in the adult population ranges from 80% to 120%.¹¹

vWF antigen assay was based on the changes in turbidity of a microparticle suspension. The antigen-antibody reaction occurs after the mixing and leads to agglutination of the latex microparticles. The agglutination increased the turbidity of the reaction medium. This was reflected by increased absorbance measured using photometry. The normal range observed during this study was from 60% to 150%.¹² All reference ranges for the haemostatic parameters were validated.

PT is a screening test for coagulation disorders in the extrinsic system domain and common pathways (Factors II, V, VII, and X). The normal range during this study was from 11.5 s to 13.5 s. PT and APTT are used as global screening procedure.¹³ The normal range for APTT was from 25 s to 35 s (established in the local laboratory using normal controls).

Platelet indices

Platelet index tests were conducted using the automated Hematology Analyzer Sysmex XE 2100 (Kobe, Japan). The mean platelet volume (MPV) is a measurement of the average size of platelets whereas platelet distribution width (PDW) is used to determine the variation of platelet size.^{14,15} MPV is known as a marker of platelet function, which means large platelets contain more dense granules and produce more thromboxane A₂.¹⁶ The normal platelet count ranges from $150 \times 10^9/L$ to $400 \times 10^9/L$.¹¹ MPV and PDW were represented as femtolitre (fL) and percentage (%), respectively. The normal range for MPV and PDW were 9–17 fL and 9–13 fL, respectively (locally validated).³⁴

Three-coloured analysis of platelet activation through flow cytometry

Flow cytometry uses the hydrodynamic principles focusing on presenting cells to a laser.¹¹ The samples were incubated with fluorochrome (fluorescein isothiocyanate [FITC], phycoerythrin [PE], and peridinin-chlorophyll [PerCP])-conjugated monoclonal antibody for platelet marker activation, i.e. CD62P-PE, PAC-1-FITC, and CD61-PerCP. The platelets were stained and tested using FACS control flow cytometer (BD Bioscience, San Jose, USA) based on the manufacturer manual.

The flow cytometry analysis for platelet activation markers is a challenging method and requires skills/correct technique at all levels from pre- to post-analytical stages. The procedure should be strictly performed to prevent platelet activation and

aggregation, especially during blood collection. In this study, the methods for platelet activation analysis followed the expert's recommendations, with careful consideration on the pre-analytical factors that might contribute to analytical errors. These challenging and methodological aspects of platelet flow cytometry analysis were previously highlighted by various authors.^{11,17} Optimisation of the method had been performed in our laboratory using normal healthy volunteers and activated samples.¹⁸

Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 13.0 (SPSS Inc, Chicago, IL). Independent t-test was used to compare mean for data with normal distribution, whereas Mann–Whitney test was performed for skewed data.

Results

Plateletpheresis donors

The mean frequency of donation was 12 times with the highest frequency in 32 donations and the lowest in 5

donations (Figure 1). Plateletpheresis donations for these donors were conducted using Haemonetics cell separator (MCS+ and MCS 3p).

Comparison of natural anticoagulant proteins

The natural anticoagulant proteins tested in this study were protein C, protein S, and AT activity assays. Protein C ($p = 0.293$) (Table 1), protein S ($p = 0.447$) (Table 2), and AT activity ($p = 0.951$) (Table 1) differences between regular plateletpheresis donors and healthy controls were statistically insignificant.

Comparison of procoagulant protein (vWF antigen)

Difference on the mean vWF antigen between the two groups was insignificant ($p = 0.923$) (Table 1). The mean vWF antigen for plateletpheresis donors and healthy controls was 92.7% and 93.3%, respectively. However, in this study, one donor had high vWF factor antigen (175%).

Comparison of platelet count, MPV, and PDW

Results showed no significant difference on the platelet count ($p = 0.248$), MPV ($p = 0.738$) (Table 1) and PDW

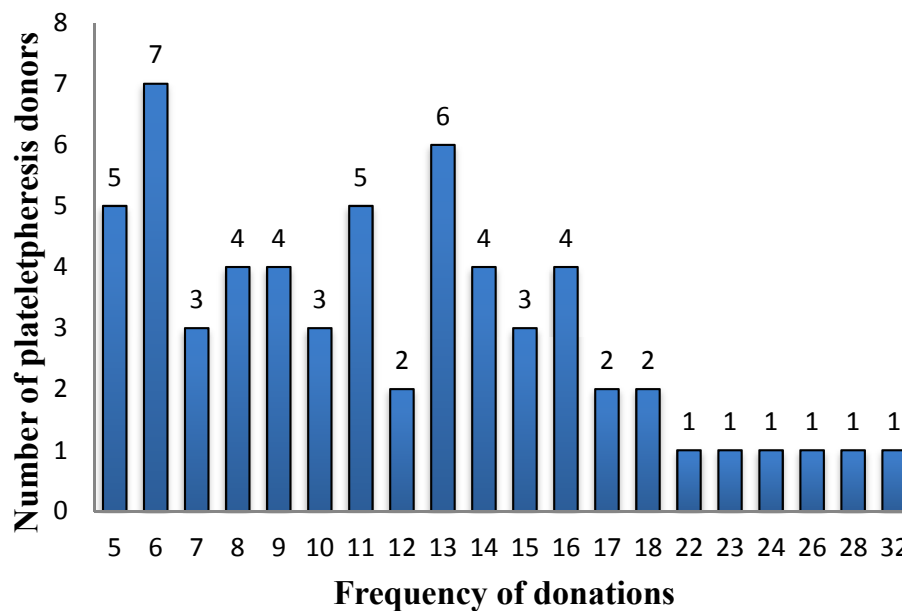


Figure 1: Frequency of plateletpheresis donations.

Table 1: Protein C, antithrombin activity, vWF, MPV, platelet count, and APTT of plateletpheresis donors and healthy controls.

Variable	Mean (SD)		<i>t</i> statistic [†] (df)	<i>p</i> -value*
	Plateletpheresis donors (n = 41)	Controls (n = 58)		
Protein C activity %	152.5 (42.09)	143.8 (39.27)	−1.058 (97)	0.293
Antithrombin activity %	104.9 (12.69)	104.8 (9.06)	−0.061 (97)	0.951
Von Willebrand Factor antigen %	92.7 (34.45)	93.3 (28.45)	0.097 (97)	0.923
MPV (fL)	9.96 (0.77)	10.01 (0.76)	0.335 (97)	0.738
Platelet count ($\times 10^9/L$)	267.95 (54.83)	254.51 (43.51)	2.137 (40)	0.039
APTT	25.89 (2.16)	25.93 (2.29)	0.093 (99)	0.926

vWF: von Willebrand Factor; MPV: mean platelet volume; APTT: activated partial thromboplastin time.

**p*-value is significant when <0.05 .

[†]Independent *t*-test.

Table 2: Protein S activity, platelet count, PDW, and PT of plateletpheresis donors and healthy controls.

Variable	Median (IQR)		Z statistic [†]	p-value*
	Plateletpheresis donors (n = 41)	Controls (n = 58)		
Protein S activity	111.0 (40)	101.5 (28)	-0.760	0.447
Platelet count	248 (54)	258 (96)	-1.154	0.248
($\times 10^9/L$)	11.30 (2.05)	11.3 (1.65)	-0.263	0.793
PDW (fL)				
PT	11.20 (0.95)	11.40 (1.03)	-2.383	0.017

PDW: platelet distribution width; PT: prothrombin time.

*p-value is significant when <0.05 .

[†]Mann-Whitney test.

($p = 0.793$) (Table 2) between plateletpheresis donors and healthy controls. However, the difference of the mean baseline platelet count (before the first-time plateletpheresis procedure) and current platelet count (pre-donation) ($p < 0.05$) was statistically significant among regular plateletpheresis donors. The mean current platelet count was lower ($254.5 \times 10^9/L$) than the mean baseline platelet count ($267.9 \times 10^9/L$). However, the levels were within the normal range for platelet count.

Comparison of PT

PT results between plateletpheresis donors and healthy controls were significantly different ($p < 0.05$) (Table 2). The median PT for plateletpheresis donors and healthy controls was 11.40 s and 11.20 s, respectively.

Comparison of APTT

No significant difference of APTT was observed between regular plateletpheresis donors and healthy controls with p-value of 0.926 (Table 1). The mean APTT for plateletpheresis donors and healthy controls was 25.89 s and 25.93 s, respectively.

Comparison of PAC-1

The analysis showed significant difference of PAC-1 between regular plateletpheresis donors and healthy controls ($p < 0.05$) (Table 3). Among the plateletpheresis donors, only one participant had PAC-1 level of $>12.5\%$, while none from the control group had the level higher than the reference range (0.1–12.5%).¹⁹

Comparison of CD62P

The analysis showed a significant difference of CD62P between regular plateletpheresis donors and healthy controls ($p < 0.05$) (Table 3). Among the plateletpheresis donors, 11 participants (55%) had CD62P level of $>4.7\%$, while 1 participant from the control group had the level higher than the reference range (0–4.7%).¹⁹

Discussion

This study investigated some of the haemostatic parameters in 61 regular plateletpheresis donors who underwent repeated plateletpheresis procedures consecutively for ≥ 5 times within the last 18 months prior to the blood sampling. The results were compared with 78 healthy controls that had never donated whole blood or any other blood components. The median PT was 11.20 s in plateletpheresis donors, which was slightly shorter than the reference ranges; however, the results were within the reference ranges for healthy controls. One study concluded the CT prolongation after the donation was due to the exposure of anticoagulant, which was present in the additive of the blood donation bag. Yilmaz et al. using similar method showed no significant difference in this parameter.⁹ PT and APTT prolongations in patients are usually associated with risk of bleeding, except those with lupus anticoagulant where patients are usually asymptomatic or showing thromboembolic manifestations.

Shortened PT or APTT is usually considered as laboratory artefacts during blood collection; however, evidences showed that in some cases, a short APTT may reflect a hypercoagulable state.²⁰ In one study on short APTT found among routine laboratory samples, differences in various test parameters (e.g. PT; factors V, VIII, XI, and XII; vWF antigen; and collagen-binding activity) and levels of pro-coagulant phospholipids were statistically significant.²⁰ This suggests the presence of complex hypercoagulant milieu that could contribute to a thrombotic risk. Shortening of PT in the present study might not reflect the true in vivo process of the donors, unless other evidences of hypercoagulation state are observed. Furthermore, the whole process of coagulation system was not included to prove this finding. The activation of coagulation during blood sampling and processing must be excluded. FVII activation is one of the factors that can be induced by cold temperature. So far, the clinical significance of shortened PT is unknown.

A study reported the haemostatic imbalance can occur after plasma exchanges and plateletpheresis procedures, including protein C and AT reduction.⁸ However, differences in protein C ($p = 0.293$), protein S ($p = 0.447$), and AT ($p = 0.951$) activities between the regular plateletpheresis donors and healthy controls were insignificant in this study. In another related study, the level of protein C and plateletpheresis donations did not show any significant difference. However, these respective studies were conducted by comparing the protein C level during pre- and post plateletpheresis donations.^{9,21} Haemostatic changes are expected to occur immediately post procedure

Table 3: PAC-1 and CD62P platelet activation markers (%) among regular plateletpheresis donors and control participants.

Variable (%)	Median (IQR)		Z statistic [†]	p-value*
	Plateletpheresis donors (n = 20)	Controls (n = 20)		
PAC-1	1.92 (3.30)	1.16 (0.88)	-2.868	0.004
CD62P	4.90 (7.16)	1.74 (0.83)	-2.789	0.005

*p-value is significant when <0.05 .

[†]Mann-Whitney test.

due to extracorporeal activation and are not clinically harmful.

The insignificant difference of protein C level in this study might be explained by Mannucci et al.'s study on the measurement of protein C (activity and antigen) after 40 plasma exchanges in 26 patients treated for various disorders. A reduction of protein C level immediately after the plasma exchange was parallel with the volume of the plasma exchange. However, at 24 h post-plasma exchange, the protein C level did not differ significantly compared to the pre-exchange levels. This was noted after doing serial measurement of the protein C levels up to 24 h. This study suggested that the reduction was transient and the level had reversed to normal after 24 h.²² In plateletpheresis, the amount of plasma collected is small and hence not affecting the measurement even among the regular plateletpheresis donors.

The reduction of protein S and AT might be a transient phenomenon, whose levels returned to normal after a period of time. Kobayashi and Yilmaz reported no significant difference of protein S and AT; however, the comparison were conducted at pre- and immediately post plateletpheresis donations.^{9,22} Based on the present study, repeated plateletpheresis donations did not affect the natural anticoagulant proteins, such as protein C, protein S, and AT detected during the time interval before the next platelet donation. These findings might suggest that repeated plateletpheresis procedures are generally safe and do not significantly alter the main anticoagulation proteins.

vWF is stored in secretory granules of endothelial cells and platelets, known as Weibel-Palade bodies and α -granules, respectively.²³ Vessel injury will lead to the secretion of vWF, which promote platelet adhesion. Here, vWF antigen was studied in regular plateletpheresis donors as one of the markers of hypercoagulation. Previous studies revealed that fibrin monomers and procoagulant proteins such as vWF activity were increased following automated apheresis leading to a possibility of hypercoagulable state.^{24,25} These studies were conducted during and immediately after apheresis (plasma and cytappheresis) as well as in plasma-exchanged patients. In contrast to this study, the present study revealed no significant difference ($p = 0.923$) on the vWF antigen between regular platelet donors and healthy controls.

An increase in vWF level following vessel injury is expected to occur during the donation procedure. vWF is also a known risk for vascular thrombosis. However, it could be a transient increase that will be normalised later. When measuring at resting state, the level would be normal as observed in this study. However, the present study found two participants with high vWF levels, one from the plateletpheresis group and the other from the healthy control group. Therefore, regular plateletpheresis procedure causes no increase in the vWF antigen and likely a transient increase with no pathological significance.

Reduction of platelet count is a well-known complication of plateletpheresis donation.¹ In this study, regular plateletpheresis donors showed no significant difference in the platelet count when compared with the healthy controls ($p = 0.248$). This finding agreed with the previous report that the reduction of platelet count was only transient. A study conducted by Dettke et al. (1998) on endogenous

thrombopoietin after automated plateletpheresis showed that the platelet count dropped up to >50% (from pre-donation count) immediately after the donation. They reported that on day 4, the platelet count increased to almost 100% in males and >90% in females.²⁶

The current platelet count before platelet donation and the baseline platelet count (first platelet count) were significantly different among regular plateletpheresis donors ($p = 0.039$). The platelet count was lower in the current count (mean, $254.5 \times 10^9/L$) compared to the baseline count (mean, $267.9 \times 10^9/L$). The sustained decrease in platelet counts were also observed in the retrospective study on multiple, regular plateletpheresis donation conducted by Lazarus et al. They traced the databases of regular plateletpheresis donations over a 4-year period and the first and last platelet counts of regular donors were compared. They found the frequency of donation correlated directly with the decrement of the platelet count.⁶ Even though the reduction of platelet count was significant, the platelet level was still within the normal range and donors are still safe to donate platelet regularly.

During activation, platelets undergo morphological changes including pseudopodia formation. With the increasing numbers and sizes of pseudopodia, MPV and PDW would be affected. In a study conducted on patients with confirmed platelet activation, a significant increase of MPV and PDW was observed.²⁷ The authors suggested that MPV and PDW could predict the haemostatic activation and might be potential markers for the early diagnosis of thromboembolic risk. MPV and PDW could be useful parameters to assess platelet activation and may indirectly predict the potential pre-thrombotic state.

In this study, MPV and PDW were analysed among the regular plateletpheresis donors and compared with those among healthy controls. The MPV and PDW were insignificantly different between regular plateletpheresis donors and healthy controls ($p = 0.738$ and $p = 0.793$, respectively). Based on these results, the median of MPV and PDW for plateletpheresis donors were within the normal range. A study on platelet activation during pre- and post-automated plateletpheresis donations conducted by Karadogan and Undar revealed a significant decrease of CD62P-positive platelet percentage, circulating activated platelets, and reduced aggregation responses to three agonists (ADP, collagen, and ristocetin) in donors after plateletpheresis procedures.²⁸ This implicated that plateletpheresis procedures did not cause increased platelet activation. Platelet activation using flow cytometry had no significant effects in pre-apheresis and post-apheresis samples from those donors. The study was performed by comparing the types of cell separators (Spectra LRS, MCS+, Amicus).²⁸

Significant increase in the expression of CD62P ($p = 0.005$) and PAC-1 ($p = 0.004$) were recorded. The median CD62P and PAC-1 were 4.90% and 1.92% in plateletpheresis donors and 1.74% and 1.16% in healthy controls, respectively. The median CD62P was slightly higher than the normal range (reference range, 0.0–4.7%).¹⁹ Nonetheless, even though a significant difference for PAC-1 was observed, the level was within the reference range (0.1–12.5%).¹⁹ Based on these findings, mild platelet activation

was detected in regular plateletpheresis donors at the interval period prior to the next plateletpheresis procedure.

Previous studies found a relationship between platelet activation and thromboembolic disease. Hagberg et al. demonstrated that activated platelets can be detected in the bloodstream passing growing thrombi at a wall shear rate characteristic of moderately stenosed arteries. In their study on CD62P, PAC-1, CD 63, and CD42a, they suggested activation of circulating platelets and correlated it with the thrombus formation.²⁹ In acute cerebral infarction, the levels of plasma CD62P were obviously higher than those of the healthy controls.³⁰ The level of plasma CD62P was higher in severe cerebral infarction compared to mild type, which would progressively decline on the following days after infarction.

The findings from the present study showed probably a mild activation of platelets by slightly increased CD62P in regular plateletpheresis donors. The effect may not be harmful unless with the presence of other risk factors such as concomitant medical illness, e.g. hypertension, hyperlipidaemia, etc.

Protein C and protein S deficiencies are usually associated with venous thrombosis. Fortunately, anticoagulant proteins such as protein C and protein S deficiencies are rare in the population worldwide. Increased platelet activity is associated with arterial thrombosis, such as myocardial infarction and ischaemic stroke.^{31,32} Therefore, regular plateletpheresis donors should be monitored for medical comorbidities throughout their active donation period.

In conclusion, prolonged effect on coagulation deficit was not observed, although mild platelet activation may be possible among regular plateletpheresis donors. Repeated plateletpheresis donation is generally a safe procedure as long as the available guidelines are adhered strictly. Careful donor selection and eliminating suspected high-risk donors are recommended. This includes metabolic diseases, such as hypertension and diabetes mellitus. Continuous health monitoring should be practiced with regular check-ups on regular plateletpheresis donors. This study recruited donors with less intensive plateletpheresis donations, which can be considered as one of the important limitations (Figure 1). Another limitation is the limited number of samples used in this study. A larger-scale study preferably on intensive plateletpheresis donor is suggested. Further studies and case reports on haemostatic effects of plateletpheresis procedure should be explored in the future.

Ethical approval

Ethical approval was obtained from the Human Research Ethics Committee, Universiti Sains Malaysia (USM/KK/PPP/JEP/219.3 (9)) and Ministry of Health Ethical Committee (NMRR-08-1577-3065). Informed consent was obtained prior to data collection.

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Authors' contributions

WZA conceived and designed the study, provided the research materials, and is the corresponding author. THTM and LSH participated in data collection and analysis. THTM wrote the initial draft of the article.

Conflict of interest

The authors have no conflict of interest to declare.

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