Bahari M[1], Yusoff N M[1], Abd Karim F[2], Hashim H[1], Abdel-Hamid A Z[1]

[1]Advanced Medical and Dental Institute, Universiti Sains Malaysia, Penang Malaysia.
[2]National Blood Centre, Kuala Lumpur, Malaysia.

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Correspondence: Narazah Mohd. Yusoff
Email: Narazah@amdi.usm.edu.my

The study of platelet activation in platelet concentrates prepared by four types of apheresis machines

Background: Platelets play an important role in forming a mechanical plug that seals a site of vascular injury during normal haemostatic response to vessel wall injury. The demand for platelets keeps on increasing in Malaysia due to several therapies which use platelet concentrates. Platelet concentrates are prepared by automated apheresis machines. The quality of the obtained platelet concentrates can be affected by several events during preparation by different methods. **Objectives**: This study aimed to compare the outcome of four types of apheresis machines; Trima, Amicus, MCS+ and Cobe Spectra in terms of their platelet activation status and, in addition, to propose the best method to achieve the good quality of platelet concentrates. **Methodology:** The study included 120 samples of platelet concentrates supplied by the National Blood Centre, Kuala Lumpur, Malaysia. Activated platelets were identified by using monoclonal antibodies against platelet surface glycoprotein. The monoclonal antibodies included CD61 (specific to glycoprotein), CD62 (to evaluate platelet surface changes) and PAC-1(conformational change of the GPIIb-IIIa complex).The markers of platelet activation were detected by flow cytometry. **Results:** Platelets obtained from Amicus showed significantly higher activation of platelets. However, platelet activation was minimal in platelet concentrates obtained from Cobe Spectra and Trima as compared to other apheresis machines. **Conclusion:** The best quality of platelet concentrates with least activated platelets can be achieved easily from platelets collected using Cobe Spectra and Trima machines.

Keywords: Platelets, Platelet concentrate, Apheresis machine.

INTRODUCTION

Progress has been made in our basic understanding of platelet functions and their utilizations in various bleeding disorders {1}. In 1910, raising the platelet count in thrombocytopenic patients by transfusing the whole blood was the first successful attempt. Continuous and accelerating progress in techniques to separate platelets from whole blood revolutionized the field of component therapy. Platelet concentrates are available for transfusion in two forms such as random donor platelets (RDP), [platelet rich plasma-platelet concentrate (PRP-PC) and buffy coat poor-platelet concentrate (BC-PC)] and single donor platelets (SDPs), collected from voluntary thrombocytapheresis donors with the help of an automated cell separator. The automated apheresis machine has been the standard of platelet support in the National Blood Centre in Malaysia since 1980. However, the quality of the platelet concentrates prepared by automated apheresis machine method has not yet been reported in Malaysia. The quality of platelet concentrates is affected by several factors including different preparation methods which may induce a variable degree of platelet activation {2}. Studies have shown that a high degree of platelet activation in the platelet concentrates is found to be associated with a reduced response to agonist stimulation {3, 4}, a low survival of transfused platelets {5, 6}, and prolonged in vitro bleeding time {7}. Platelet activation in platelet concentrates could contribute in platelet storage lesion which may have an impact on their structure and function in normal haemostatic**.**This study was designed to compare the outcome of four types of apheresis machines; Trima, Amicus, MCS+ and Cobe Spectra in terms of their platelet activation status to ensure effective platelet transfusion at the National Blood Center, Malaysia.

MATERIALS AND METHODS

The study included 120 samples of platelet concentrates which were taken from apheresis bags provided by the National Blood Centre (NBC), Kuala Lumpur, Malaysia. The samples were prepared by the trained and qualified staff under standardized protocols with informed consents of volunteer donors by fulfilling the blood service donation criteria in the component laboratory of the National Blood Centre, Kuala Lumpur. Each sample from all four automated apheresis machines was tested for platelet activation at interval of pre, after 2 hours resting period, day 1 and day 2 of storage. Thirty samples of platelet concentrates were taken from each apheresis machine. Activated platelets were identified using monoclonal antibodies against platelet surface glycoproteins. These monoclonal antibodies included CD61 (specific to glycoprotein), CD62 (to evaluate platelet surface changes) and PAC-1(conformational change of the GPIIb-IIIa complex). The markers of platelet activation were detected by flow cytometry.

RESULTS

The multivariate tests indicated that there were significant differences in platelet activation levels across time *($p<0.001$)* and there were also significant interaction between time and machine *($p<0.001$).* Only the Amicus machine showed the differences. The other three machines belonged to the same group. This was consistent with results given by pair-wise comparisons which showed significant differences among method, time and platelet activation.

No significant differences were observed in platelet activation amongst platelets obtained from Cobe Spectra,

Trima and MCS+. Even though activation of platelets occurred, platelets activations were minimal in platelet concentrates which were obtained from Cobe Spectra and Trima as compared to the other apheresis machines.

Fig. 1: Example of CD62 expression in platelet concentrates prepared from Amicus.

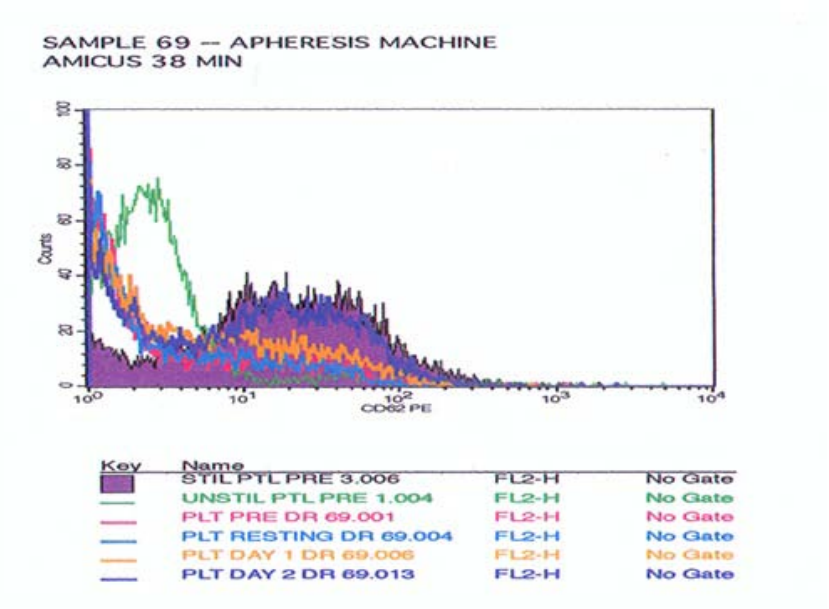


Table I: Description of platelet activation in platelet prepared by Apheresis machines.

Descriptive Statistics

	machine	Mean	Std. Deviation	N
pre	mcs	1.9557	1.17890	23
	amics	14.6676	14.53079	25
	trim	1.8381	1.97891	26
	spectra	.9959	.71862	17
	Total	5.2351	9.59247	91
resting	mcs	4.0683	2.91626	23
	amics	20.7596	16.68303	25
	trim	6.4792	4.64229	26
	spectra	5.4582	5.37812	17
	Total	9.6023	11.65737	91
d1	mcs	23.4783	15.38122	23
	amics	31.6396	17.89545	25
	trim	21.9065	11.57272	26
	spectra	20.3888	13.21607	17
	Total	24.6942	15.19894	91
d2	mcs	38.0400	16.35478	23
	amics	27.0196	15.15809	25
	trim	23.2441	9.72902	26
	spectra	23.3765	9.83818	17
	Total	28.0457	14.37889	91

Table II: Comparison of platelet activation levels prepared by four different apheresis machines.

Time	MCS+ (*n*= 23) Mean (SD)	Amicus (*n*= 25) Mean (SD)	Trima (*n*= 26) Mean (SD)	Spectra (*n*= 17) Mean (SD)	*F* stat *(df)*[a]	*P* value[a]
Pre test	1.9557(1.17890)	14.6676(14.53079)	1.8381(1.97891)	0.9959(0.71862)	7.00 (6.4, 184.4)	<0.001 (sig.difference betweeen group & across the time)
Settling	4.0683(2.91626)	20.7596(16.68303)	6.4792(4.64229)	5.4582(5.37812)		
Day 1	23.4783(15.38122)	31.6396(17.89545)	21.9065(11.57272)	20.3888(13.21607)		
Day 2	38.04009(16.35478)	27.0196(15.15809)	23.2441(9.72902)	23.3765(9.83818)		

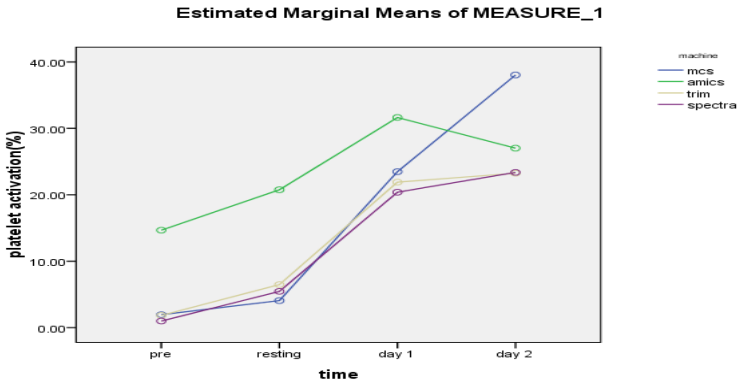
[a] repeated measures ANOVA (Time & Group Interaction Effect) [H_o: There is no difference in platelet activation levels prepared using different apheresis machines]

Table III: Multiple comparisons
Measure: MEASURE_1 Bonferroni

(I) machine	(J) machine	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
MCS+	Amics	-6.6361(*)	2.42674	.045	-13.1881	-.0840
	Trim	3.5186	2.40427	.882	-2.9728	10.0099
	Spectra	4.3307	2.68645	.663	-2.9225	11.5839
Amicus	Mcs	6.6361(*)	2.42674	.045	.0840	13.1881
	Trim	10.1546(*)	2.35269	.000	3.8025	16.5067
	Spectra	10.9667(*)	2.64038	.000	3.8379	18.0956
Trima	Mcs	-3.5186	2.40427	.882	-10.0099	2.9728
	Amics	-10.1546(*)	2.35269	.000	-16.5067	-3.8025
	Spectra	.8121	2.61975	1.000	-6.2610	7.8852
Spectra	Mcs	-4.3307	2.68645	.663	-11.5839	2.9225
	Amics	-10.9667(*)	2.64038	.000	-18.0956	-3.8379
	Trim	-.8121	2.61975	1.000	-7.8852	6.2610

Based on observed means.
* The mean difference is significant at the .05 level.

Fig. 2: Comparison of platelet activation in various platelet concentrates prepared by four types of Apheresis machine.



DISCUSSION

The quality of platelet concentrates prepared by apheresis machines has so far never been officially reported by the National Blood Centre of Malaysia. Several studies have shown that the levels of platelet activation in platelet concentrates prior to storage are influenced by the method of processing {2}. The results from this study confirm earlier reports that the levels of platelet activation are influenced by the method of preparation. Moreover, our results indicate that there is an increase in CD62 expression in platelets prepared by apheresis machines although the levels of platelet activations were different.

Platelet samples were tested as pre-collection samples, samples after 2 hour resting period, day 1 and day 2 of the storage. The platelets obtained from Amicus showed significantly higher platelet activation. Immediately after collection in pre-collection samples and day 1 of storage, platelets obtained from Amicus showed more activation than the platelets obtained from other apheresis machines. We also observed a higher expression of CD62 on resting platelets obtained by Amicus. Platelet apheresis by certain cell harvesters may induce platelet activation, probably because of sheer stress between platelets and artificial surfaces of limited biocompatibility {8}. Our results obviously show that immediately after preparation, platelets obtained from Amicus are more activated than platelets obtained from other apheresis machines. This is most probably due to platelets resuspension process. Platelets obtained from Amicus are reported to be more activated than platelets obtained from MCS^+ and Cobe Spectra during the first 4 hours after cessation of the apheresis {9}. The technology of Amicus is different when compared to the other apheresis machines. In Amicus, platelets are first collected and then separated in a small bag within the centrifuge. Once plateletpheresis is completed, the hyper concentrated platelets are resuspended in plasma before being transferred to the final storage bag. The manual shaking, the different durations of platelets shaking and the resuspension process involve vigorous handling. This is the reason of higher platelet activation in platelet concentrates obtained from Amicus.

At day 2 of storage, platelets obtained from Amicus show slightly decreased levels of platelet activation but platelets obtained from other apheresis machines keep on increasing to some extent during storage. In this study, platelets activation in platelet samples which were obtained by apheresis machines could not be observed until day 5 of storage but recent studies have revealed that the platelets obtained by Amicus express more P-selectin and secrete more soluble CD40L than units obtained with Trima on day 1, 5 and 7 of storage {10}.

No significant differences were observed in platelet activation amongst platelets obtained from Cobe Spectra, Trima and MCS+, although activation of platelets occurred during storage. At pre-sample collection, platelets obtained from these three apheresis machines showed lower levels of platelet activation. In platelets obtained by MCS+ showed increased levels of platelet activation at day 2 of storage. This is probably because the MCS+ have the longest donation time and needle time as mentioned earlier by the work of Picker et al., {11}.

No significant difference was observed in platelet activation between platelets obtained from Cobe Spectra and Trima machines as found in a previous work by Tynngard et al., {12}.

CONCLUSION

Our finding has shown that the best quality of platelet concentrates with least activated platelets is better than from those platelets concentrates which are collected using Cobe Spectra and Trima machine.

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