

Original Article

Collection Efficacies of Double Dose Platelet by Blood Cell Separators

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Background: New generation blood cell separators enable multiple unit collection of platelets from the one eligible donor in a single procedure. The advantages of this procedure are to maximize donor resources and platelet inventory as well as to minimize the possibilities of transfusion associated diseases and production costs. **Objectives:** The objective of this study is to evaluate the collection efficacy of two blood cell separators, the Amicus version 3.1 (AM) and the Trima Accel version 5.0 (TA) for double dose plateletpheresis (DDP). In vitro platelet quality and in vivo platelet transfusion effectiveness were studied. In addition, donor safety and satisfaction were evaluated. **Method:** Double dose platelets were collected from 45 eligible donors for the target of 6.5×10^{11} by two blood cell separators with interval of 4 weeks. The products were measured for platelet yield, residual white blood cells (WBC), pH, and volume. Platelets were transfused to patients and transfusion effectiveness was measured by the corrected count increments within 1 hour (CCI-1) and 24 hour (CCI-24) after transfusion. **Results:** The Amicus version 3.1 produced a significantly higher platelet yield with lower residual WBC counts than that of the Trima Accel version 5.0 (7.24 vs. 6.87×10^{11} platelets per DDP, $p = 0.001$; 0.34 vs. 0.82×10^6 WBCs per single donor platelet after manually separation into 2 single donor platelets, $p = 0.022$). For leukoreduction, all of the single platelet units had fewer than 5×10^6 WBCs per unit. The mean amount of whole blood processed by the AM were significant higher than that of the TA ($3,574.09$ vs. $3,355.67$ mL, $p < 0.01$). The AM and the TA were insignificantly different regarding to the processing time, collection efficiency, platelet volume, and anticoagulant volume. No severe donor reaction was observed during or after the procedure. Only a few differences in donor satisfaction were found between the two systems. The AM was more preferable by donors than the TA, especially regarding the use of the pressure cuff, which facilitated the draw cycles. **Conclusions:** The effectiveness of double dose plateletpheresis and the quality of platelet product in vitro by new generation blood cell separators has been revealed by this study. Double dose plateletpheresis was performed efficiently and safely by both cell separators. All of the platelet units passed international standard requirement.

Key Words : ● Plateletpheresis ● Double dose plateletpheresis ● Double dose platelets

J Hematol Transfus Med 2013;23:121-8.

Received January 17, 2013. Accepted May 22, 2013.

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Introduction

In recent years, pooled random whole blood derived platelet concentrates have been widely replaced by single donor apheresis platelet (SDP) collected by blood cell separators¹⁻⁴. This is based on the assumption that one unit of SDP contains the same platelet yield as 4-6 units of whole blood derived platelets^{5,6}. The advantages of the SDP includes the ability to reduce multiple donor exposures and the transmission of transfusion diseases^{7,8}. While the demand for SDP has been increasing, the retention of voluntary and eligible blood donors have been facing more difficult. Double dose plateletpheresis (DDP) enables the collection of 2 SDPs from one eligible donor in a single donation. DDP can provide an adequate supply of platelets in the context of limited human resources and shrinking donor populations. This helps reducing the problem of donor retention, minimizing production costs, and the risks associated with allogeneic transfusion⁹⁻¹². The objective of this study was to evaluate the collection efficacy of two blood cell separators for DDP, with respect to in vitro and in vivo platelet quality, donor safety and satisfaction.

Materials and Methods

Donors

Donors were voluntary repeated donors from blood bank, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. This study was approved by the Ethics Clearance Committee of Human Right Related to Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital. Forty-five blood donors were enrolled in the study from July 2010 to January 2011. The sample size was calculated with 95% confident interval from variances in collection efficiency of previous data¹³. All donors were required to meet the eligible criteria defined by the American Association of Blood Bank (AABB)¹⁴, and to give a written informed consent prior to be included in the study. The donors were selected for first DDP with the target yield of 6.5×10^{11} for both instruments if their pre-procedure platelet count were $\geq 250 \times 10^3/\mu\text{L}$. At least 4 weeks

later, each donor was switched to the other blood cell separator for the second donation. Each donor answered a questionnaire for the subjective assessment of each donation satisfaction. Satisfaction scores were recorded from 1 to 10. Donor reactions including perioral tingling, pain at needle site and dizziness were recorded during and after the procedure.

Blood Cell Separator

Two blood cell separators were evaluated in this study. The first one was the Amicus version 3.1 (AM), a continuous flow blood cell separator manufactured by Fenwal, IL, USA. The AM had a belt-shaped chamber with two compartments, a separation chamber and a collection chamber. In the separation chamber, the platelet (PLT) rich plasma was separated from red blood cells (RBC) and white blood cells (WBC) and then pumped into the collection chamber, where PLT was concentrated. The PLT poor plasma and RBCs were returned to the donor. At the end of the procedure, the PLTs were resuspended manually by shaking and then transferred with the PLT poor plasma to a storage bag. The second one, the Trima Accel version 5.0 (TA), a continuous flow blood cell separator was manufactured by Terumo BCT, Lakewood, USA. The TA had a single stage channel with a leukoreduction system (LRS) chamber. Anticoagulated whole blood was pumped into the channel and separated into components. PLTs were collected by passing through the LRS chamber which trapped WBCs into a storage bag. The PLT poor plasma and RBCs were returned to the donor.

Laboratory Measurements

Donor safety

Peripheral blood samples were collected for complete blood count (CBC) before and after each procedure for donor safety determination. Pre-procedure blood samples were collected immediately from sample pouches. Post-procedure samples were collected immediately after the procedure by discarding the first 5 mL of blood before collection in order to prevent sample dilution.

In vitro study

Product samples were collected after overnight storage of platelet before separation into 2 storage bags. Samples were diluted 1:10 with 0.9% normal saline (NSS) and then platelet yield were measured by automated blood cell counter (Sysmex KX-21). Platelet pH was determined on the day of transfusion at room temperature with a pH meter (Fisher Accumet Model 20). Platelet yield were calculated using the formula:

Platelet yield (10^{11}) = Product platelet count ($\times 10^3/\mu\text{L}$) x Product volume (mL) x Conversion factor (1,000). Residual WBCs were determined by the flow cytometry (FACSort, Becton Dickinson, San Jose, CA, USA) with propidium iodide staining and fluorescence beads (LeucoCOUNT kit, BD Biosciences, San Jose, CA, USA).

Collection efficiencies

Collection efficiencies were calculated using the formula:

Collection efficiency (%) = [Platelet yield / Total platelets processed (TPP)] x 100

TPP = [pre + post PLT count / 2] x total blood processed (mL) x conversion factor (1,000)

Total blood volume processed =
blood volume processed – anticoagulant volume

In vivo transfusion effectiveness

Transfusion effectiveness was assessed by the corrected count increment (CCI) within 1 hour and 24 hours (CCI-1, CCI-24) after transfusion. Platelet products were transfused to patients who had no conditions which would potentially reduce the CCI. They were antibody to human leukocyte antigen (anti-HLA), hypersplenism and disseminated intravascular coagulation. The platelet transfusion reaction was observed and recorded by nurses.

Statistical Analysis

The two tailed paired t-test was performed using SPSS for Windows, version 18 (IBM SPSS Inc., Chicago IL.). Numerical data was tested for normal distribution

with the Kolmogorov-Smirnov test. Ordinal data was compared with Wilcoxon Signed Ranks Test. Nominal data was compared with the McNemer test. A p-value of ≤ 0.05 was considered significant.

Results

Forty-five blood donors, 31 males and 14 females, were included in this study and a total of 90 donations were evaluated. The donor characteristics were shown in Table 1. Table 3 showed the comparison of apheresis parameters of the AM and TA procedures. The quality of platelet products by DDP were summarized in Table 4. All of the units collected by both instruments had a mean pH of 6.87 which pass the minimal standard requirement (≥ 6.2). Pre-procedure and post-procedure hematologic parameters of donor were summarized in Table 2 and Table 5, respectively.

Adverse reactions during and after the procedures were mild and limited to citrate associated reactions, as shown in Table 7. Only perioral tingling was observed and no severe adverse reaction was found in this study. More donor reaction were noted for the Amicus than those of the Trima. Donor satisfaction mean scores were summarized in Table 6.

The CCIs of 10 transfusions from 3 patients were assessed for transfusion effectiveness. Most of the SDP were transfused for therapeutic purpose in bleeding patients rather than prophylactic purpose. It was observed that CCI at 1 hour increased in 4/10 transfusions and the patients showed partially effective responses to transfusions, notably by the bleeding was slowing down. Only 1 transfusion had increased CCI at 24 hours.

Table 1 Donor characteristics (N = 45)

Characteristics	Mean \pm SD
Age (yrs)	39.27 \pm 8.34
Weight (kg)	74.25 \pm 13.93
Height (cm)	167.91 \pm 7.52

Table 2 Pre-donation hematologic parameters of donors

Parameters	Mean \pm SD		<i>p</i> -value
	Amicus version 3.1	Trima version 5.0	
Pre- procedure			
Platelet counts (x10 ³ /μL)	339.18 \pm 33.72	334.33 \pm 33.84	0.250
Hematocrit (%)	42.20 \pm 3.33	42.69 \pm 2.77	0.097
Hemoglobin (g/dL)	13.80 \pm 1.24	13.97 \pm 1.05	0.080
White blood cells (x10 ³ /μL)	6.86 \pm 1.77	7.11 \pm 1.80	0.107
Red blood cells (x10 ⁶ /μL)	4.99 \pm 0.54	5.05 \pm 0.49	0.065

Table 3 The comparison of apheresis parameters

	Mean \pm SD		p-value
	Amicus version 3.1	Trima version 5.0	
Whole blood processed (mL)	3,574.09 \pm 420.76	3,355.67 \pm 321.49	< 0.01*
ACD-A used (mL)	393.18 \pm 41.15	400.63 \pm 38.65	0.199
Processing time (min)	73.49 \pm 12.18	69.16 \pm 14.95	0.093
Collection efficiencies (%)	86.00 \pm 6.21	85.31 \pm 8.27	0.637
Collection rate (PLTs $\times 10^{11}/\text{min}$)	0.1 \pm 0.02	0.1 \pm 0.02	0.636

*Significant difference

Table 4 The quality of platelet products collected by DDP

	Standard requirement	Mean \pm SD		p-value
		Amicus version 3.1	Trima version 5.0	
Platelet volume (mL)	-	418.22 \pm 55.9	411.45 \pm 11.74	0.421
Platelet yield ($\times 10^{11}$)	≥ 6.0	7.24 \pm 0.53	6.87 \pm 0.60	0.001*
Residual WBC ($\times 10^6$)	≤ 8.0	0.34 \pm 1.30	0.82 \pm 1.94	0.022*

*Significant difference

Table 5 Post-donation hematologic parameters of donors

Post- procedure	Mean \pm SD		p-value
	Amicus version 3.1	Trima version 5.0	
Platelet counts ($\times 10^3/\mu\text{L}$)	197.44 \pm 27.01	216.82 \pm 33.36	< 0.01*
Hematocrit (%)	39.96 \pm 3.26	41.18 \pm 3.73	0.008*
Hemoglobin (g/dL)	13.11 \pm 1.16	13.47 \pm 1.28	< 0.01*
White blood cells ($\times 10^3/\mu\text{L}$)	7.97 \pm 1.80	8.01 \pm 2.53	0.897
Red blood cells ($\times 10^6/\mu\text{L}$)	4.75 \pm 0.53	4.89 \pm 0.61	0.011*

*Significant difference

Table 6 Donor satisfaction mean scores

Question	Mean		p-value
	Amicus version 3.1	Trima version 5.0	
Instrument satisfaction	8.87	7.96	0.008*
Donation time	8.16	7.53	0.258
Process satisfaction	9.09	8.56	0.057
Information providing by staff	9.07	8.78	0.161
Overall satisfaction	9.11	8.51	0.022*

*Significant difference

Table 7 The percentage of donor reaction observed during and after DDP

Symptoms	Donor reaction (%)		
	Amicus (version 3.1)	Trima (version 5.0)	p-value
Perioral tingling	77.8	55.6	0.052
Pain at needle site	15.6	11.1	0.774
Dizziness	6.7	4.4	1.000

Discussion

DDP were collected from the same donors for the same target yield by both the AM and TA. The pre-procedure donor platelet count for each instrument was not statistically significant, (AM, $339.18 \pm 33.72 \times 10^3/\mu\text{L}$ vs TA, $334.33 \pm 33.84 \times 10^3/\mu\text{L}$, $p = 0.25$), as shown in Table 2. Pre-procedure donor CBC were also not statistically significant. This reduced the possible bias from donors which might affect the results.

Some major safety concerns for DDP were the donor's post-procedure blood cell loss and anticoagulant associated adverse events. The post-procedure hematologic parameters, including PLT count, Hct, Hb, RBC count, from the AM collection were significantly lower than those of the TA. Only WBC count was not statistically significant. However, all of the parameters were within the normal range. The AM returned 425 mL of 0.9% NSS as the process of return while no NSS was reinfused from the TA. The post-procedure samples were taken immediately after reinfusion. This may cause the decline in hematological parameters from the AM than those of The TA. However, none of the donors had post-procedure PLT counts $< 100 \times 10^3/\mu\text{L}$ and they all passed the international recommendations. Therefore, DDP can be

performed efficiently and safely by both instruments.

The comparison of apheresis parameters of both instruments showed no significantly different, except the AM processed significantly more whole blood than the TA ($3,574.09 \pm 420.76 \text{ mL}$ vs. $3,355.67 \pm 321.49 \text{ mL}$, $p < 0.01$). The processing time of the AM and TA which was the time from the start until completion of reinfusion, were not significantly different (73.49 ± 12.18 vs. $69.16 \pm 14.95 \text{ min}$, $p = 0.093$). However, the AM needed extra time for product transfer while the TA collect the product into the final storage bag during the collection. Moreover, the AM needs an additional 10-15 minutes' handling time for manual shaking and resuspension of PLTs prior to transfer the product to the final storage bag. The collection efficiency (CE) of the AM and TA, which was the percentage of harvested platelet against total platelet processed, were not significantly different ($86 \pm 6.21\%$ vs. $85.31 \pm 8.27\%$, $p = 0.637$).

For product characteristics, the AM had significantly higher mean PLT yield than those of the TA (7.24 ± 0.53 vs. 6.87 ± 0.60 , $p = 0.001$), even though the CE, processing time, collection rate and PLT volume were similar. This may be explained by the fact that the AM processed significantly higher whole blood volume

than the TA. However, after separation of PLTs into 2 SDP, all of SDPs from both instruments obtained $> 3 \times 10^{11}$ PLTs which passed the AABB standard. Therefore, both instruments were capable of DDP.

According to Food and Drug Administration (FDA) guidelines¹⁵, the residual WBC in double dose platelet cannot exceed 8×10^6 . It was observed that the mean residual WBCs were higher in the PLTs from the TA than those of the AM ($0.34 \pm 1.30 \times 10^6$ vs. $0.82 \pm 1.94 \times 10^6$, $p = 0.022$). However, most of them had WBC less than 8×10^6 , within the FDA guidelines. Only one DDP from the AM did not pass the FDA guideline. The donor was a healthy man with available large vein access, and there was no alarm or other problems detected during the process. The residual WBC count was 8.7×10^6 . However, after separation into 2 SDP, each unit passed the AABB standard ($< 5 \times 10^6$ WBCs per unit).

Concerning donor reactions, there was no significant difference between both instruments. Donor reactions were mild and no serious adverse effect was found during or after any of the procedures. This may be related to the fact that the donors were repeated whole blood donors and had the experience of donating apheresis components. Regarding to hypocalcemic symptoms, all of the reactions resolved rapidly by decreased whole blood flow rates or oral calcium supplementation. Concerning the subjective assessment of donor satisfaction, the donors reported the minor different satisfaction between both instruments in terms of instrument satisfaction and overall satisfaction. Donors preferred to donate DDP by the AM than the TA (95.6% VS 64.4%, $p = 0.001$). It might have been due to the pressure sleeve which facilitated drawing cycles during the process of the AM. However, all of the donors were willing to donate DDP again for both instruments.

This study was a preliminary evaluation of the platelet transfusion effectiveness from SDP prepared by DDP. The patient sample sizes were too small to conclude the effectiveness of SDP products. Some transfusions

(4/10) showed increased CCI-1 and partially effective responses to transfusions. However, some transfusions showed ineffective response by unchanged bleeding or occurrence at the new site. They might be explained by the patients' unpredictable underlying conditions which might potentially reduce the CCI. Therefore, it was important to be aware that the poor CCI values were not necessarily attributable to poor quality of the product alone, but may be due to unstable and variable clinical status of an individual patient¹⁶.

The other advantage of DDP is the reduction of production costs. Separation of platelet products into 2 SDP from one donation can reduce about 50% of the production costs. DDP could reduce the cost of labour, disposable sets, infectious disease testings, and quality assurance.

Conclusion

It was observed from our study that DDP can be performed efficiently and safely by both the AM and TA. The higher platelet yield and lower residual WBC were observed in the AM than those of the TA. On the other hand, the AM processed more whole blood volume. Both instruments had similar processing time and collection efficiency. There was no significant difference in donor safety but the donors have more satisfaction with the AM than the TA.

Acknowledgement

Special thanks to our donors for their invaluable cooperation, and to the staff of blood bank, Ramathibodi Hospital who gave us their friendships and valuable technical support throughout the study.

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การเปรียบเทียบประสิทธิภาพการเก็บ Double Dose Platelet โดยเครื่องแยกส่วนประกอบของเลือดอัตโนมัติ

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บทคัดย่อ เครื่องแยกส่วนประกอบของเลือดในปัจจุบันสามารถเก็บเกล็ดเลือดได้ในปริมาณมากจากการบริจาคเพียง 1 ครั้ง ในผู้บริจาครายเดียวที่ผ่านเกณฑ์การคัดเลือก ทำให้มีประโยชน์ในการเพิ่มปริมาณเกล็ดเลือดสำรองในคลังเลือด สามารถลดความเสี่ยงการติดเชื้อจากการรับเลือด และยังสามารถลดต้นทุนการผลิต **วัตถุประสงค์** การศึกษาค้นคว้าเพื่อประเมินประสิทธิภาพของเครื่องแยกส่วนประกอบของเลือดอัตโนมัติ Amicus version 3.1 (AM) และ Trima Accel version 5.0 (TA) ในการเก็บเกล็ดเลือดแบบ Double dose plateletpheresis (DDP) โดยศึกษาคุณภาพเกล็ดเลือดที่เก็บได้ในหลอดทดลองและติดตามประสิทธิภาพของเกล็ดเลือดเมื่อนำไปให้แก่ผู้ป่วย รวมไปถึงศึกษาความปลอดภัยและความพึงพอใจของผู้บริจาค **วิธีการ** ผู้บริจาคจำนวน 45 รายที่ผ่านเกณฑ์การคัดเลือก ผู้บริจาค บริจาคเกล็ดเลือดแบบ DDP โดยกำหนดจำนวนเกล็ดเลือดที่ต้องการเก็บเป็น 6.5×10^{11} เซลล์ต่อการบริจาค 1 ครั้ง และเว้นระยะการบริจาคอย่างน้อย 4 สัปดาห์ เมื่อบริจาคเสร็จในแต่ละครั้งมีการสอบถามความพึงพอใจผู้บริจาค บันทึกปฏิกิริยาที่เกิดขึ้นระหว่างการบริจาค ระยะเวลาในการเก็บเกล็ดเลือด ตรวจคุณภาพของเกล็ดเลือดที่เก็บ ได้แก่ ปริมาณเกล็ดเลือด ปริมาณเม็ดเลือดขาวปนเปื้อนและ ติดตามผลของการให้เกล็ดเลือดแก่ผู้ป่วย โดยเจาะเลือดผู้ป่วยที่เวลา 1 และ 24 ชั่วโมงหลังจากให้เกล็ดเลือด คำนวณค่า Corrected count increment (CCI) **ผลการศึกษา** เครื่อง AM และเครื่อง TA สามารถเก็บเกล็ดเลือดแบบ DDP ได้ตามเกณฑ์มาตรฐาน โดยเครื่อง AM สามารถเก็บเกล็ดเลือดได้มากกว่าเครื่อง TA (7.24×10^{11} vs. 6.87×10^{11} platelet per DDP, $p = 0.001$) และมีปริมาณเม็ดเลือดขาวปนเปื้อนน้อยกว่าอย่างมีนัยสำคัญ (0.34 vs. 0.82×10^6 WBCs per unit, $p = 0.022$) เมื่อแบ่งเป็น single donor platelet 2 ถุง พบว่าทุกถุงมีปริมาณเม็ดเลือดขาวปนเปื้อนน้อยกว่า 5×10^6 ตัวต่อเกล็ดเลือดหนึ่งถุง ค่าเฉลี่ยของปริมาณเลือดหมุนเวียนที่ใช้ในการเก็บเกล็ดเลือดของเครื่อง AM มากกว่าเครื่อง TA อย่างมีนัยสำคัญ ($3,574.09$ vs. $3,355.67$ mL, $p < 0.01$) ค่าเฉลี่ยของระยะเวลาในการเก็บ ประสิทธิภาพในการเก็บเกล็ดเลือด ปริมาตรของเกล็ดเลือด และปริมาณสารกันเลือดแข็งที่ใช้ของทั้งสองเครื่องไม่แตกต่างกันอย่างมีนัยสำคัญ พบว่าผู้บริจาคมีความพึงพอใจเครื่อง AM มากกว่า เนื่องจากมี pressure sleeve ที่แขนบอกรังสีในการดึงและคืนเลือด ไม่พบอาการข้างเคียงชนิดรุนแรงระหว่างและหลังการบริจาค **สรุป** เครื่อง Amicus (V3.1) และ Trima Accel (V5.0) สามารถเก็บเกล็ดเลือดแบบ Double dose plateletpheresis ได้อย่างมีประสิทธิภาพและให้ความปลอดภัยแก่ผู้บริจาค คุณภาพของเกล็ดเลือดที่เก็บได้ ผ่านเกณฑ์มาตรฐานระดับนานาชาติ

Key Words : ● Plateletpheresis ● Double dose plateletpheresis ● Double dose platelets

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2556;23:121-8.