Quality Assessment of Platelet Concentrates Prepared after Whole Blood Overnight Storage

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Abstract—**The present study focuses to assess the in vitro effect on quality of platelet concentrates (PCs) prepared from overnight-held whole blood (WB). Method: Thirty four units of WB were stored overnight at 20 to 24oC and processed on the following day, and 30 units of WB were processed immediately. Result: The volume of PCs from overnight-held WB on Day 1 was significantly lower. On the same day, the PCs were significantly lower in platelet count but by Day 3 and Day 5, the difference was disappeared. PCs made from overnight-held WB had a significantly higher total red blood count on Day 1, Day 3 and Day 5, and for total white blood count was significantly higher on Day 5. pH was significantly lower on Day 1, Day 3 and Day 5. Sterility test for both groups of PCs give negative results. Conclusion: PRP-derived PCs from overnight-held WB have significantly different in vitro variables as from freshly processed WB. However, based on one-sample t-test comparison with the quality requirement of PCs, the use of the PCs from overnight-held WB was possible as the results of volume, platelet count and pH were significantly higher than the quality requirement and specification.**

Index Terms—**platelet concentrates, quality assessment, overnight storage**

I. INTRODUCTION

Blood components are commonly prepared from whole blood (WB) as soon as possible after blood collection. The maximum storage period before components preparation is often within 8 hours at 20 to 24°C [1]. There is increasing interest in extending this period to eliminate the needs for multiple transport runs from collection sites to the component preparation facility [2]. It also allows the component preparation units on one rather than multiple shift in the production of components from the WB [3]. Therefore, the staffs for blood component preparation are basically needed only during routine working hours and the workload can be evenly distributed over time. Thus, it provide very efficient production of blood component by avoiding periods of waiting for WB to be supplied from different blood collection sites [1].

Determination of platelet quality is important to confirm that the platelet concentrates (PCs) meet the requirements defined by national and international standards and to evaluate the effect of PCs transfused to the thrombocytopaenic patient [4]. The quality of PCs is affected by the preparation method of the PCs, the plastic material of the storage bag, the storage conditions including duration of storage, the storage temperature, type of anticoagulant used, the concentration of PCs in the bag and the agitation [5] and [6].

The possibility of the PCs prepared from overnight-held WB to be used for transfusion to thrombocytopaenic patients can be detected by measurements of several in vitro platelet quality parameters [4]. The present study focuses on in vitro quality assessment of PCs prepared from overnight-held WB by analyzing the volume, platelet count, total red blood count (TRBC), total white blood count (TWBC), pH and sterility. This study compared in vitro quality of platelet-rich plasma (PRP)-derived PCs from fresh versus overnight-held WB. In addition, this present study also compares the PCs prepared from overnight-held WB with the quality requirement and specification stated by National Blood Centre.

II. MATERIALS AND METHODS

A. Study Design

This study was performed in National Blood Centre, Kuala Lumpur. The design of the study was prospective, controlled study. Test samples of WB with volume between 429mL to 525mL in triple blood bags citrate-phosphate-dextrose-adenine (CPDA) anticoagulant with saline-adenine-glucose-mannitol (SAGM) solution (Terumo) were stored overnight (18 hours) at room temperature (20 to 24°C). For the control in this study, the PCs were prepared from the WB within eight hours after the collection.

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B. Platelet Concentrates Preparation

PCs in this study were prepared by PRP-derived PCs method. The temperature of refrigerated centrifuge (Jouan KR4i) was set at 20°C. The WB was centrifuged by using a "light" spin (2200 rpm for 8 minutes). PRP was expressed into the satellite bag intended for platelet storage. The PRP bags were centrifuged at 20°C using a "heavy" spin (3800 rpm for 10 minutes). The platelet-poor plasma was expressed into the second transfer bag without disturbing the platelet button. The volume of the PCs was adjusted to 63 to 68 mL with plasma. The PCs bags were left stationary, with the label side down, at room temperature for one hour to rest. PCs were stored with continuous agitation in a quarantine platelet incubator (Helmer) at 20-24°C.

C. Quality Assessment of Platelet Concentrates

Volume, platelet count, TRBC, TWBC and pH of the PCs were measured on Day 1, Day 3 and Day 5. Sterility of PCs was measured on Day 5 to detect the presence of bacterial growth.

- *Volume determination:* The volumes of PCs were determined by subtracting the weight of empty bag from that of full bag. To convert weight to volume, resultant weight was divided by 1.03 specific gravity of PRP-PC.
- *Platelet count, TRBC and TWBC:* Platelet count, TRBC and TWBC were determined by using standard calibrated haematology analyzer (Beckman Coulter LH750). The haematology analyzer was ensured to set up for the full blood count test. 2 ml of PCs was filled in 4 ml plain tube and mixed properly. The cassettes were loaded with the PCs in vacutainer plain tubes. The cassettes were placed firmly and securely into the loading bay of the haematology analyzer. The analyzer was automatically begins cycling the cassette. After the analyzer cycles the samples, the samples result was reviewed on the workstation.
- *PH determination:* pH for PCs was measured by using pH meter (Mettler Toledo Delta 320 pH). The electrode of pH meter was placed in PCs and swirled the solution. The pH reading had to be stabilized before the pH result of PCs taken. When the reading was freezing, the pH of PCs was recorded.
- *Sterility test:* Sterility was performed on Day 5 of the PCs shelf-life. Bacterial growth examination was performed by using automated blood culture system (Organon Teknika). The plastic flip-top from the culture bottle was removed and disinfected with an alcohol pad. 10 ml of PCs was obtained by using syringe with aseptic technique and it was transferred into the culture bottle. The PCs sample had to transfer into the anaerobic culture bottle first before putting in aerobic culture bottle. So that any oxygen trapped in the syringe was not be transferred to the anaerobic bottle. The bottle was loaded into the automated blood culture system by scanned the bottle barcode label. The cultured bottle had to be incubated in the automated blood culture system for seven days. If there was positive culture, light was flashing from the automated blood culture system.

D. Statistical Analysis

Comparisons were performed on PCs from fresh versus overnight-held WB using a two-sample t-test at a 95% confidence level. Besides, comparisons were also performed on PCs from overnight-held WB versus quality control requirement by National Blood Centre by using one-sample t-test. A p-value of less than 0.05 was used to indicate a significant difference.

III. Result

Thirty four bags of WB with volume of mean (SD) 452.76 (2.22) mL (range, 436 mL to 493 mL) were stored overnight at 23°C (range, 22°C to 24°C). The actual holding times before processing were 18:06 (00:21) hours for the overnight-held units (range, 14:50 to 22:00 hours).

Thirty bags of WB with average volume of 458.37 (2.25) mL (range, 435 mL to 485 mL) were prepared for PCs freshly after collection and the temperature were maintained at 23°C (range, 22°C to 24°C). The actual holding times before processing were 5:16 (00:19) hours for the fresh units (range, 2:10 to 8:32 hours).

Quality of PCs processed from the overnight-held WB versus freshly prepared PCs was analyzed and result of the study was presented in Table I. On Day 1, p-value was 0.019 ($p<0.05$), therefore there was sufficient evidence to indicate that the volume of PCs prepared from overnight-held WB was lower than the freshly prepared PCs on Day 1. Since p-value was 0.021 ($p<0.05$), there was sufficient evidence to indicate that the platelet count for PCs from overnight-held WB was lower than the freshly prepared PCs on Day 1. PCs made from overnight-held WB had a significant higher TRBC on Day 1 (p-value was <0.001), Day 3 (p-value was 0.001) and Day 5 (p-value was 0.023) versus freshly prepared PCs ($p<0.05$). Since p-value was 0.039 on Day 5, there was sufficient evidence to indicate that the TWBC for PCs from overnight-held WB is higher than the freshly prepared PCs on Day 5.

PCs made from overnight-held WB had a significantly lower pH on Day 1, Day 3 and Day 5 versus freshly prepared PCs. All Day 5 PCs for both group of samples showed no bacterial growth in the aerobic (FA) and anaerobic (FN) cultured bottle after seven days incubation period in automated culture system (Organon Teknika).

Quality of PC from overnight-held WB was compared with quality requirement stated by National Blood Centre by using one-sample t-test. Result of the study was presented in Table II. Since all p-value between PCs from overnight-held WB versus the quality requirement stated by National Blood Centre showed less than 0.05, there was sufficient evidence to indicate that volume, platelet count, TRBC, TWBC and pH of the PCs prepared from overnight-held WB was higher than the quality

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requirement and specification stated by National Blood Centre [7].

TABLE I. QUALITY ASSESSMENT OF PRP-DERIVED PC PREPARED FROM WB THAT PROCESSED FRESHLY VERSUS OVERNIGHT HELD

Variable	Freshly prepared (*n*=30)[a]	Overnight storage (*n*=34)[a]	Mean diff. (95% CI)	*t*-test	*p*-value[b]
Volume (mL)					
Day 1	67.80 (1.58)	66.82 (1.67)	0.98	2.40	0.019
Day3	64.79 (1.56)	63.96 (1.90)	0.84	1.19	0.061
Day 5	61.40 (1.47)	60.92 (1.71)	0.49	1.23	0.228
PC ($x\ 10^9$/unit)					
Day 1	111.57 (19.66)	99.21 (21.88)	12.36	2.37	0.021
Day 3	96.40 (18.70)	97.97 (21.99)	-1.57	-0.31	0.761
Day 5	92.40 (18.74)	90.35 (20.38)	2.05	0.42	0.679
TRBC ($x\ 10^6/\mu L$)					
Day 1	0.01 (0.01)	0.02 (0.01)	-0.01	-3.98	<0.001
Day 3	0.01 (0.01)	0.02 (0.01)	-0.01	-3.62	0.001
Day 5	0.02 (0.01)	0.03 (0.03)	-0.01	-2.34	0.023
TWBC ($x\ 10^9$/unit)					
Day 1	0.04 (0.02)	0.06 (0.06)	-0.02	-1.95	0.057
Day 3	0.03 (0.01)	0.04 (0.03)	-0.01	1.48	0.146
Day 5	0.03 (0.01)	0.04 (0.03)	-0.01	-2.14	0.039
pH					
Day 1	7.62 (0.08)	7.48 (0.07)	0.14	7.26	<0.001
Day 3	7.59 (0.13)	7.37 (0.13)	0.22	6.80	<0.001
Day 5	7.48 (0.20)	7.27 (0.28)	0.21	3.40	0.001

[a]Mean (SD), [b] Two-samples independent *t*-test

IV. DISCUSSION

The use of an overnight-held of WB donations at room temperature before processing has introduced several operational advantages to blood centers. With the overnight-held WB practice, component manufacturing, including platelet preparation, can be facilitated, logistically, with related cost savings. In addition, PCs could be prepared from every unit of WB regardless of the distance from the collection site to the processing centre, and PCs could be processed on a single shift, which would reduce operating costs. Therefore, the number of shipments of WB from collection sites can be significantly reduced and the workload in the laboratory can be distributed evenly over time as the component preparation will be needed only during working hours.

A few studies had addressed the effect of an extended WB storage time at room temperature before component preparation with the PRR method. According to Thibault *et al.* [8] delaying PCs processing up to 24 hours did not affect their in vitro measures. Walther-Wenke *et al.* [9] stated that a potentially detrimental consequence of a prolonged ambient hold period could increase the bacterial growth in the WB unit. However, the presence of leucocytes in the WB during the ambient hold period allows natural defense mechanisms to auto-sterilize the WB, as long as the leucocytes are removed during processing.

TABLE II. QUALITY ASSESSMENT OF PRP-DERIVED PC PREPARED FROM WB OVERNIGHT HELD VERSUS QUALITY REQUIREMENT STATED BY NATIONAL BLOOD CENTRE

Variable	Quality requirement (specification)	Overnight storage (*n*=34)[a]	Mean diff. (95% CI)	*t*-test	*p*-value[b]
Volume (mL)					
Day 1	50 (10)	66.82 (1.67)	23.77	6.82	<0.001
Day3		63.96 (1.90)	12.16	3.96	<0.001
Day 5		60.92 (1.71)	3.14	0.92	0.004
PC ($x\ 10^9$/unit)					
Day 1	>60	99.21 (21.88)	10.45	39.21	<0.001
Day 3		97.97 (21.99)	10.07	37.97	<0.001
Day 5		90.35 (20.38)	8.68	30.35	<0.001
TRBC ($x\ 10^6/\mu L$)					
Day 1	0	0.02 (0.01)	11.40	0.02	<0.001
Day 3		0.02 (0.01)	11.55	0.02	<0.001
Day 5		0.03 (0.03)	7.12	0.03	<0.001
TWBC ($x\ 10^9$/unit)					
Day 1	<0.2	0.06 (0.06)	-13.59	-0.16	<0.001
Day 3		0.04 (0.03)	-32.77	-0.16	<0.001
Day 5		0.04 (0.03)	-27.23	-0.16	<0.001
pH					
Day 1	>6.5	7.48 (0.07)	77.07	0.98	<0.001
Day 3		7.37 (0.13)	38.10	0.87	<0.001
Day 5		7.27 (0.28)	16.24	0.77	<0.001

[a]Mean (SD), [b] One-sample *t*-test

Platelet product quality control validates the preparation spin times and speeds, and the storage conditions. Therefore, platelet products could be tested at the time of expiration or at the time of use [10]. This study focuses on the assessments of the quality of PCs prepared from overnight-held WB at room temperature on Day 1, Day 3 and Day 5 to know whether the quality of the platelets is affected by the hold conditions. Three

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continuous assessments days of PCs with two days interval allow the pattern of quality changes being determined in this study.

In study by Moroff *et al.* [11] and also Dijkstra-Tiekstra *et al.* [3], there was no significant difference in the mean volume either PCs prepared from overnight-held WB or freshly prepared PCs. In the present study, all units of PCs were fulfilled the quality control criteria of volume. The difference was significant on Day 1 between freshly prepared PCs and overnight-held WB with the *p*-value was 0.019 ($p<0.05$). Plasma-suspending volume affected the volume of PCs prepared from overnight-held WB on Day 1 giving the volume of PCs significantly lower than the freshly prepared PCs. However, this significant difference does not have any deleterious effect to maintain the pH throughout the storage period by its buffering action. Platelets prepared from overnight-held WB and freshly prepared PCs were stored in donor plasma, which serves as a buffering agent. These PCs were typically suspended in 50 to 70 ml plasma to maintain pH [12]. Therefore, the platelet-suspending volume was maximized to increase buffering capacity while maintaining as little volume as possible, to minimize the risk of volume overload of the recipient's circulatory system [13].

Volume of PCs reduced throughout the storage duration on Day 1, Day 3 and Day 5 was because of approximately 4 mL of PCs being taken from the platelet bags for the purpose of platelet count, TRBC, TWBC and pH determination. Method of sampling by using sterile tubing welder can maintain and control the sterility of the sample taken as well as the remaining of PCs in the platelet bag.

Platelet count on Day 1 for PCs from overnight-held WB was significantly lower than the freshly prepared PCs. The explanation for lower platelet yield is the assessment of platelet count on Day 1 for PCs prepared from overnight-held WB was on the same day with their preparation day. Therefore, there were disaggregation of platelet-platelet aggregates that had formed during centrifugation and preparation of PCs. Aggregated platelet that had no time to break down would be excluded from sampling for the platelet count assessment [14]. Different situation for the freshly prepared PCs that were prepared on the day before (Day 0), it had longer duration of storage at room temperature with agitation on Day 1, allowing the platelet-platelet aggregates to break down into single platelet suspensions. However, during storage, through Day 3 and Day 5, the platelets count for PCs prepared from overnight-held WB apparently similar effect with freshly prepared PCs.

As shown in the article describing RBC and BC-PCs quality [2], a higher level of platelet count was present in BC-PCs with 27% higher content in the BCs from overnight-held PCs. More recent study shows a 33% higher platelet concentration was determined from PCs prepared from overnight-stored WB versus freshly prepared PCs ($p<0.05$) [15]. In other study done by Dijkstra-Tiekstra *et al.* [3] it can be seen that freshly prepared PCs had the lowest platelet count and PCs prepared from overnight-held WB had the highest platelet counts.

All the previous studies stated were used active cooling devices to cool the blood to 20 to 24°C after collection, but in the present study, which did not use active cooling devices, may be the reasons of giving the lower platelet count result for the PCs from overnight-held WB versus freshly prepared PCs. Cooling devices is use to control the degree of temperature to the desirable one to ensure that the WB is neither too warm for too long, nor does get it too cold. However, it is lack of study to consider whether the temperature control provided by active cooling devices will affect the platelet count produced by collected WB [14].

Even the platelet count on Day 1 was significantly lower for PCs from overnight-held WB, the platelet count was significantly higher than the quality control requirement stated in Guidelines for Clinical and Laboratory Personnel, National Blood Centre [7]. Therefore, the platelet count in single unit of PCs prepared from overnight-held WB is sufficient to be use for the transfusion in thrombocytopaenic patient.

TRBC in this study showed significant higher on Day 1, Day 3 and Day 5 for PCs from overnight-held WB versus freshly prepared PCs. The random PCs were prepared from WB by soft-spin to separate the red cells from the PRP and high-spin to separate platelet from the PPP. The presence of red cells in PCs in this study might be caused by the flow of very little amount of red cells into the PCs transfer bag during the first separation process after the soft-spin (first spin). During the hard-spin, the residual red cells may sediment at the bottom together with the platelets. Therefore, the improper separation of PRP from the red cells concentrates after the first spin may cause the presence of residual red cells in the PRP and consequently present of the residual red cells in the PCs. The range and standard deviation in this study was wide, thus more standardization is required in the preparation of PCs, especially during the first separation, after the soft-spin.

The TWBC seem to be significantly higher on Day 5 for PCs prepared from overnight-held WB than the freshly prepared PCs. The range and standard deviation of TWBC in this study is wide, thus more standardization is required in the preparation of PCs. In a study of ambient hold of WB, significantly more WBC were counted in non-leucodepleted WB after a 12 hours ambient hold period compared with a 4 to 8 hours ambient hold [14]. Different result presented in study done by Dijkstra-Tiekstra *et al.* [3], in which there was no significant differences for TWBC count between PCs prepared from overnight-held WB and freshly prepared PCs. Other studies showed that overnight storage of WB at room temperature can reduce the risk of bacterial contamination since bacteria will be phagocytosed by WBCs [3] and [15].

Quality requirement of TWBC by National Blood Centre [7] is less than 0.2×10^9/unit. However, the mean of TWBC for Day 1, Day 3 and Day 5 for both groups of samples in this study showed less than 0.2×10^9/unit.

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Therefore, this lesser amout of WBC in PCs from overnight-held WB can reduce the risk of CMV transmission, febrile reactions and HLA immunization [16].

Data from this study showed that there were significantly lower of the pH on Day 1, Day 3 and Day 5 after overnight storage. Since the significant differences are already seen on Day 1, it is more probable that glucose is consumed and lactate is formed by the RBCs and WBCs in the WB and the BC during overnight storage. This resulting in initial reduced pH for PCs prepared from overnight-held WB compared to freshly prepared PCs. However, the pH results still appeared to be significantly higher than the specification of the quality control requirement proposed by National Blood Centre.

Decrease in pH for PCs prepared from overnight-held WB has previously been observed both for the PRP and for the BC method. A natural consequence is increased production of lactate from glucose by red cell glycolysis associated with a small drop in pH of about 0.1 unit as compared to the level in reference WB units processed within 8 hours [1]. Study done by Dijkstra-Tiekstra *et al.* [3] reported that the metabolism variables pH, glucose, and lactate showed best results for freshly prepared PCs, compared to the overnight-held WB.

Fall in pH to levels approaching 6.0 in PC stored in plasma is associated with substantial loss of viability of the platelet [13]. To assure the platelets in the product are viable and functional, the pH must not be acidic and the volume of plasma in which the platelets are suspended must be adequate to keep the pH neutral and allow for gas exchange [10]. The pH decreases during storage also depending on the stabilizer in plastic platelet storage bags and storage conditions used [13].

No bacterial growth in PCs for both groups of PCs in this study after culturing on Day 5 of the PCs. Previous in vitro studies performed by inoculating PCs with bacteria suggest that a significant number of PC experience the most rapid rate of bacterial growth on days six and seven of storage [17]. Blajchman *et al.* [18] found that when 16,290 platelet cultures were sampled on day 1 of culture, four culture-positive PCs were found. Then, an additional three platelet concentrates were found on reculturing on day 3 of storage. If the previous study only gets four culture-positive out of 16, 290 units of platelet culture, the probability in the present study to get culture-positive result is low, unless the sample size is increase to several hundred or thousand PCs units.

Transfusion of a bacterially contaminated platelet component carries an estimated risk of a clinically significant event at 1 in 25,000 transfusions [18]. Sources of bacterial contamination in PCs can be from donor bacteraemia, at collection sites by the skin flora, during processing either during separation or sampling, and contamination of blood bag either contaminated anticoagulant or leaky seal, damage tube or micro-puncture in collection bags. Preparation in an entirely closed, sterile system and the mechanism of the sterile docked, enable these study to provide a product with decreased risk of bacterial contamination.

V. Conclusion

PRP-derived PCs from overnight-held WB have significant difference in vitro variables as from freshly processed WB. However, the use of PCs from overnight-held WB was possible as the results of all variables were significant higher than the quality requirement and specification stated in Transfusion Practice Guidelines for Clinical and Laboratory Personnel released by National Blood Centre [7].

Overall, this study indicates that the in vitro quality of PRP-derived PCs, made from WB that has been stored overnight at room temperature, is at least within the quality requirement of PCs to be transfused in thrombocytopaenic patients. There were as yet limited in vivo data available, but these also suggest that overnight storage of WB is not unfavorable for the quality of PCs. In the future study, platelet function test can be performed on the PCs from overnight-held WB to assess the function status of the platelet. More in vitro and in vivo data need to be collected both before and at the time of implementation of overnight-held of WB for production of PRP-derived PCs. Decisions to implement overnight room temperature hold should also include the effect on the RBC and plasma quality and must be balanced against the logistical benefits of overnight hold.

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