

Quantitative and Qualitative Analysis of Platelet-Rich Plasma Collection Using the Haemonetics Cell Saver 5 in Open Heart Surgery

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Abstract: Many traditional autologous blood recovery systems (ABRSs) have undergone modifications to enable them to collect platelet-rich plasma (PRP). Because of the growing demand for autologous platelet gel (APG) in cardiac surgery, many open heart teams are faced with the choice of using their existing ABRS or purchasing a dedicated PRP device. This study was conducted to address the issues we had about our use of the Haemonetics Cell Saver 5 (CS5) to collect PRP during open heart surgery at our institution. PRP and platelet-poor plasma (PPP) were collected on 20 “first-time” elective open heart surgical patients. Baseline, PRP, and PPP platelet counts, as well as modified thrombelastograms (TEGs), were performed on all

study patients. The mean baseline, PRP, and PPP platelet counts were 232,450, 1,348,850, and 18,100/mm³, respectively. We found a strong positive correlation ($r = +0.7142$) between the maximum amplitude (MA) of our modified PRP TEG and the platelet count of the PRP. Using the CS5, we achieved a mean platelet multiple of greater than six times baseline, which compares favorably with the multiple produced using dedicated PRP devices. These data support the conclusion that we achieved a high platelet multiple with the CS5, and our use of a modified TEG showed that platelet function of the collected PRP was preserved. **Keywords:** autologous platelet gel, platelet-rich plasma, thrombelastography. *JECT. 2006;38:235–240*

Over the last few years, many publications have focused on the benefits of autologous platelet gel (APG) as an adjunct to surgical procedures (1–9). Many devices are currently available to collect the necessary platelet-rich plasma (PRP), from which the gel is derived. Some of these devices are designed and marketed exclusively for the collection of PRP (e.g., Magellan; Medtronic Minneapolis, MN, and Angel; Cobe Cardiovascular, Arvada, CO). Although typically more expensive per procedure, these “single-use” devices require relatively small amounts of whole blood (40–100 mL) and provide consistently high platelet counts. Many traditional autologous blood recovery systems (ABRSs) have undergone modifications to their software, optics, and disposable tubing sets, thereby enabling these “multi-use” devices to selectively collect PRP (e.g., Cell Saver 5 [CS5]; Haemonetics, Braintree MA, Cobe BRAT II; Arvada). Although more readily available in most cardiac operating rooms, these devices typically require more whole blood to be har-

vested (minimum 300–400 mL) and result in more PRP but lower platelet counts compared with dedicated devices (10). Because of the growing demand for APG in cardiac surgery, many open heart teams (including ours) are currently facing the choice of using their existing ABRS or purchasing a dedicated PRP device.

This study was conducted to assess the feasibility and to answer the following questions about the use of the CS5 to collect PRP during open heart surgery at our institution.

1. What multiple of the patient’s baseline platelet count could be attained?
2. Could that multiple be consistently and predictably achieved?
3. Is the platelet function in the collected PRP preserved?

MATERIALS AND METHODS

Study Group

Twenty elective “first-time” open heart surgical patients, between May and August 2005, were included in this study, for which institutional review board approval was obtained. Fifteen patients underwent coronary artery bypass grafting, three received an aortic valve replacement, one received a mitral valve replacement, and a mi-

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tral valve ring was placed in one patient. Patients were excluded from this study if they were having emergency surgeries, had received platelet receptor inhibitors/antagonists within 5 days of surgery, or were considered poor candidates for blood harvesting by the attending anesthesiologist (hypovolemia, hypotension, etc.).

Whole Blood Collection

After the insertion of the Swan-Ganz catheter into the right internal jugular vein or the left subclavian vein and before chest incision, each patient in the study was phlebotomized into a 450-mL CPD-A blood collection bag (Baxter Fenwal, Round Lake, IL) by gravity.

Platelet Count

For each patient in the study, three platelet count determinations (Cell-Dyne 3200 and 400; Abbott Labs, Abbott Park, IL) were performed: a baseline whole blood sample (after insertion of the Swan-Ganz catheter and before blood harvest), a processed platelet poor plasma (PPP) sample, and a processed PRP sample.

Thrombelastography

A single two-channel Thrombelastograph Model 5000 (Haemoscope Corp., Niles, IL) was used for all determinations. Thrombelastography (TEG) provides a complete graphic representation of blood coagulation, from initial pro-coagulant activation and fibrin formation, through fibrin cross-linking and clot retraction to eventual clot lysis over time. A 0.36-mL sample (whole blood, plasma, or PRP) is placed into a plastic sample cup, which is rotated 4 degrees in either direction every 4.5 seconds, with a 1-second pause between direction changes. A plastic pin is suspended freely, from a torsion wire, into the sample. The torsion wire is attached to a mechanical-electrical transducer, which converts the mechanics of clot formation into an electrical signal. The electrical signal is conducted into a microprocessor, which uses proprietary analytical software to graphically display the TEG onto a laptop computer monitor (Figure 1). From the TEG tracing, four coagulation parameters are directly measured:

1. The "R-time" is the time required from the initiation of the testing procedure until early clot formation (2-mm amplitude).
2. The "K-time" is the time it takes to go from 2-mm amplitude to a pre-determined clot firmness or strength (20-mm amplitude).
3. The " α angle" is a measure of the angle formed as the clot develops from 2 mm in amplitude to 20 mm in amplitude.
4. The "maximum amplitude (MA)" is a measurement of the ultimate strength of the clot. A higher MA value is consistent with greater clot strength.

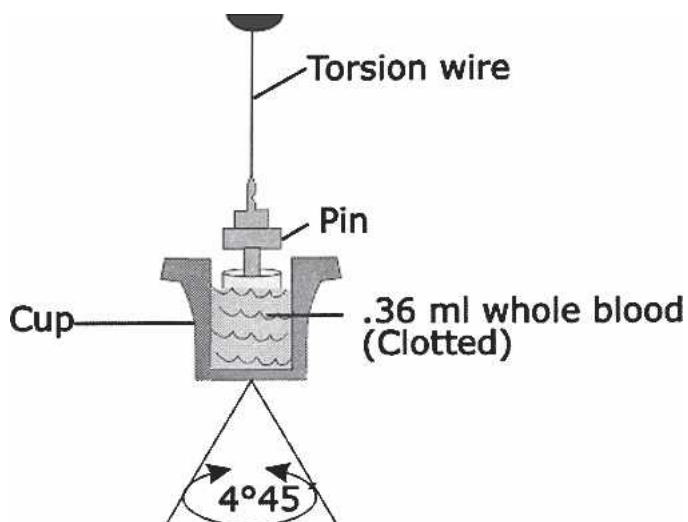


Figure 1. Thrombelastograph schematic design.

From the four directly measured TEG parameters, the Haemoscope Model 5000 calculates an overall coagulation index (CI). The normal value for the CI ranges from -3.0 to $+3.0$, with values less than -3.0 being consistent with hypo-coagulability and values greater than $+3.0$ indicating hyper-coagulability. Hyper-coagulable, normal, and hypo-coagulable TEG tracings are presented in Figure 2.

Before performing any TEGs, each channel had to first pass an internal electronic calibration as well as two biological quality controls (Level I QC "Normal" and Level II QC "Abnormal"). The lyophilized controls are made from citrated animal whole blood with the addition of stabilizers and buffer. After re-hydration with distilled water, 340 μ L of the reconstituted QC and 20 μ L of CaCl_2 are added to the test cup, and a TEG is performed. The manufacturer provides acceptable ranges for each of the four measured TEG parameters, which must be satisfied before any patient testing. After performing a Level I and Level II QC, and confirming that the results were within

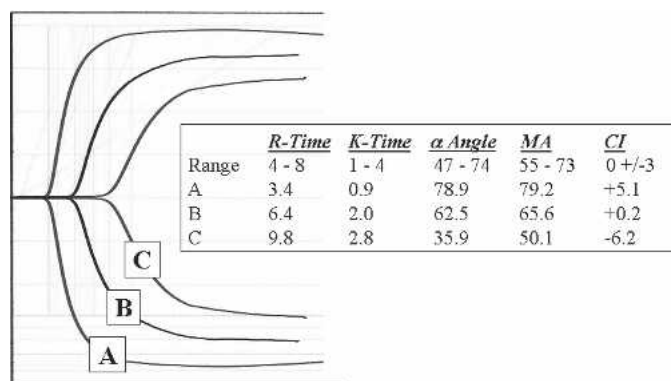


Figure 2. Hyper-coagulable (A), normal (B), and hypocoagulable TEG tracings (C).

range, the following TEGs were performed for each of the 20 study patients.

1. A PPP TEG was performed by adding 40 μL of the collected PPP to a sample cup containing 20 μL of CaCl_2 and 300 μL of the reconstituted Level 1 QC.
2. A PRP TEG was performed by adding 40 μL of the collected PRP to a sample cup containing 20 μL of CaCl_2 and 300 μL of the reconstituted Level 1 QC.

The Level 1 QC was chosen as the substrate for the TEG portion of our study because it produces consistent, predictable, highly reproducible TEG parameters. Through trial and error (adding 20, 40, 80 μL , etc.), we found that the substitution of 40 μL of PRP was enough to show its impact on the TEG (specifically the highly platelet-sensitive MA) while not exceeding the graphic range of the MA (~ 80 mm). The Level I QC plus PPP provided us with a baseline control TEG from which we could compare the PRP-substituted TEG for each patient. For each sample, the R-time, K-time, α angle, MA, and CI were recorded on our data collection worksheet.

Sequestration Technique

For all of the 20 study patients, the CS5 was set-up with a Pediatric Cell Saver 5 Bowl Set (cat. 261). Before the initiation of sequestration, the waste bag and connecting tubing (from the bowl set) was removed, stored, and replaced with the Haemonetics Sequestration Kit (cat. 244). Two modes are available for performing platelet sequestration with the CS5. In assisted sequester mode, the operator inputs pre-set programming parameters (pump speed, PPP, and PRP centrifuge speeds, "milking," etc.) and follows the prompts during processing. In manual sequester mode, the operator manually selects operating parameters and does not receive machine prompts. Before this study, it was our experience that platelet sequestration in assisted sequester mode yielded a high volume of relatively dilute PRP with platelet counts that were two to three times baseline. For this reason, we opted to use the manual sequester mode during this study. After whole blood was harvested from the patient into a CPD-A bag, it was connected to one of the two wash limbs of the CS5 circuit. The centrifuge speed was set to 4750 rpm (hard spin), and the pump speed was set to 60 mL/min at the start of the sequestration process. After pumping the "dead-space" air into the waste bag, the PPP was collected into the PPP collection bag. Using a 5-mL syringe, attached to the sampling port at the bottom of the PPP collection bag, we collected the first 3 mL of processed PPP. This volume was used for our PPP TEG sample and PPP platelet count. PPP collection continued until the red blood cell (RBC)/plasma interface advanced beyond the shoulder of the latham bowl. The system was paused, and the revolutions per minute were reduced to 3200 (soft

spin). With the centrifuge speed at 3200 rpm and the pump speed reduced to 20 mL/min, the operator re-positioned the thumb clamps to begin PRP collection. PRP collection continued until the operator collected the available PRP and ~ 5 mL of RBCs. This volume was used for our PRP TEG sample and PRP platelet count.

Data Analysis

Three platelet counts and TEG data for each study patient were recorded on a Data Collection Worksheet. This data was entered into Microstat Statistical Software (Ecosoft, Indianapolis, IN) for descriptive statistical analysis and correlation coefficients (r). Because normal baseline platelet counts can vary widely (150,000–450,000/ mm^3), we believe that the resultant PRP platelet count must be evaluated in relation to the patient's baseline platelet count, specifically as a multiple of that baseline. The platelet multiple was calculated as follows: platelet multiple = PRP platelet count/baseline platelet count.

RESULTS

The raw platelet data for the 20 patients included in our study are listed in Table 1. The mean baseline platelet count for the 20 study patients was 232,450/ mm^3 , with a range of 123,000–395,000/ mm^3 . The PPP sample had a mean platelet count of 18,100/ mm^3 , with a range of 1000–67,000/ mm^3 . The mean platelet count of the PRP was 1,348,850/ mm^3 , with a range of 506,000–2,125,000/ mm^3 .

We found a negative correlation ($r = -0.5103$) between the baseline platelet count and the platelet multiple we achieved (Figure 3). As the baseline platelet count went

Table 1. Raw platelet count data for 20 study patients.

Patient	Baseline Platelet Count (mm^3)	PPP Platelet Count (mm^3)	PRP Platelet Count (mm^3)	Platelet Multiple (PRP/Baseline)
1	210,000	5,000	1,200,000	5.71
2	216,000	11,000	506,000	2.34
3	172,000	10,000	1,424,000	8.28
4	187,000	5,000	1,411,000	7.55
5	395,000	67,000	1,314,000	3.33
6	305,000	35,000	1,196,000	3.92
7	311,000	7,000	2,079,000	6.68
8	329,000	61,000	2,125,000	6.46
9	160,000	6,000	1,195,000	7.47
10	168,000	2,000	984,000	5.86
11	336,000	48,000	1,884,000	5.61
12	130,000	1,000	813,000	6.25
13	213,000	6,000	900,000	4.23
14	268,000	50,000	1,640,000	6.12
15	202,000	29,000	965,000	4.78
16	280,000	3,000	1,335,000	4.77
17	168,000	3,000	1,200,000	7.14
18	215,000	2,000	1,811,000	8.42
19	261,000	2,000	1,642,000	6.29
20	123,000	9,000	1,353,000	11.00
Mean	232,450	18,100	1,348,850	6.11

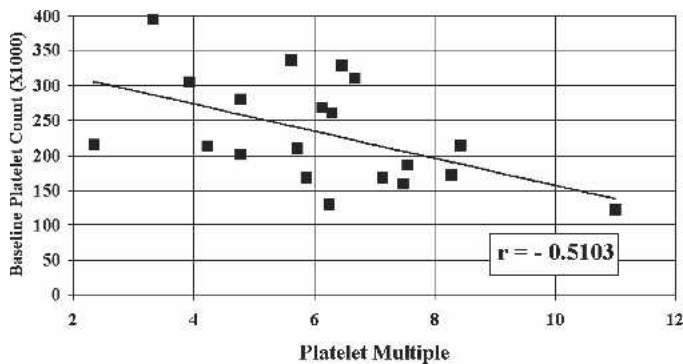


Figure 3. Relationship between the baseline platelet count and the platelet multiple.

up, the resulting platelet multiple went down. The mean platelet multiple for patients with baseline platelet counts $<200,000/\text{mm}^3$ ($n = 7$) was 7.65. For the five patients with baseline platelet counts $>300,000/\text{mm}^3$, the mean platelet multiple was only 5.20.

The strongest correlation ($r = +0.7142$) was between the absolute platelet count of the PRP and the MA of the TEG (Figure 4). As the platelet count of the PRP went up (because of higher baseline count and/or higher platelet multiple), we measured greater values for the MA of our PRP TEG samples.

In 14 of 20 patients (70%), we achieved a platelet multiple of between four and eight times baseline. In 3 of 20 patients (15%), the platelet multiple was less than four and for the remaining 3 patients (15%), it was greater than eight times baseline.

The mean platelet multiple for the four perfusionists involved in this study ranged from 4.82 to 7.99 times the baseline platelet count. As seen in Figure 5, the ranges for the four perfusionists were 4.22–8.27, 6.29–11.00, 2.34–7.47, and 3.32–6.45, respectively.

The mean volume of whole blood harvested during this study was 611 mL, with a range of 400–1100 mL. The mean volumes for PPP, PRP, and RBCs were 357 (230–600 mL), 44 (25–60 mL), and 210 mL (130–440 mL), respectively.

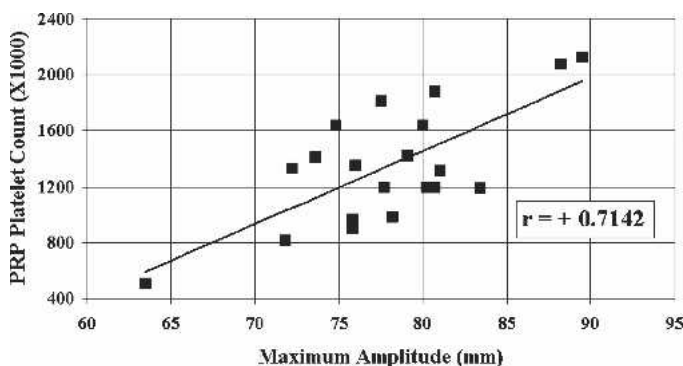


Figure 4. Relationship between the PRP platelet count and the MA of the TEG.

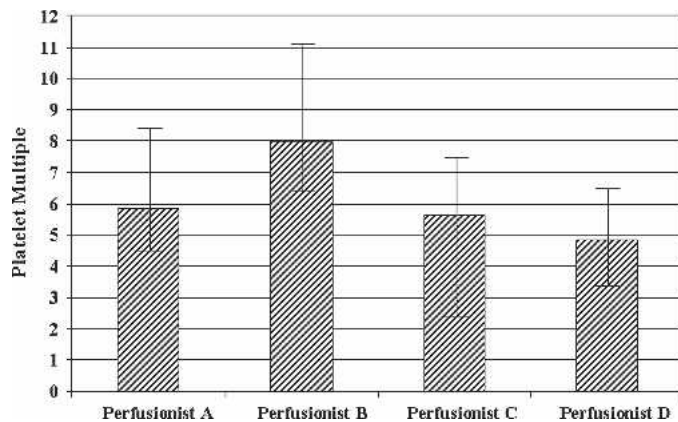


Figure 5. Perfusionist variability.

DISCUSSION

Using the CS5, we achieved a mean platelet multiple of greater than six times baseline, which compares favorably with the multiple generated with ABRs and dedicated PRP devices (10–17). Because the PRP platelet count is equal to the product of the baseline count and the platelet multiple, one would expect that a higher baseline count would predictably result in a higher PRP count. We did find this to be true (Figure 6), but because higher baseline platelet counts were also associated with lower platelet multiples, the increases in the PRP platelet counts were diminished.

Of note, however, was the variability we found in the average platelet multiple achieved by the four perfusionists and the day-to-day variability for each perfusionist. Perhaps this should not be too surprising given that the CS5 was being used in manual sequester mode. In this mode, the perfusionist must subjectively determine when to switch from “hard spin” to “soft spin,” when to reposition the thumb clamps to switch from PPP collection to PRP collection, and how far into the packed cell layer the platelet harvest will continue. Some of this variability, however, may be attributable to our finding that the plate-

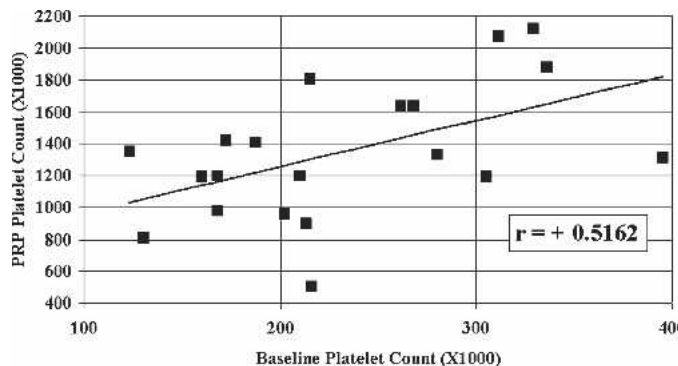


Figure 6. Relationship between the baseline platelet count and the PRP platelet count.

let multiple was also influenced by the patient's baseline platelet count. We speculated that this was a function of the 4750 rpm (hard spin) centrifuge speed used in this study. Because higher baseline platelet counts were associated with higher platelet counts in the PPP, a faster centrifuge speed may have been required to further concentrate the platelets into the "buffy coat" region. We would also expect that, over time, with a larger patient population, the perfusionist-to-perfusionist variability would inevitably narrow as we learn from experience how to manipulate the previously mentioned endpoints to maximize the platelet multiple.

To determine whether platelet function in the PRP was preserved after collection with the CS5, we used TEG. The MA parameter of the TEG tracing, a measure of clot strength, is primarily impacted by the platelet function (~80%) of the test sample and to a lesser degree by the fibrinogen level (~20%). Our use of the Level I QC plus PPP, which is relatively devoid of platelets (mean, 18,100/mm³), offered us an excellent baseline for comparison with our platelet-enriched (mean, 1,348,850/mm³) Level I QC plus PRP. Because these samples differ only in their platelet count, the difference in the MA between these two samples (PPP TEG and PRP TEG) can be attributed to differences in platelet function. As seen in Figure 7, the MA of the PRP TEG was greater than the PPP TEG for each of the 20 study patients. The smallest MA difference in the study patients was 18.2 mm, and the largest difference was 40.2 mm. As seen in Figure 8, the mean R-time, K-time, and α angle were not statistically different for the two groups. However, the mean MA for the PRP TEG samples was 156.36% greater than the mean MA for the PPP TEG samples. These data support the conclusion that, not only were we able to achieve a platelet multiple of greater than six, but the platelet function of the collected platelets was preserved.

At this time, the ideal platelet count for PRP has yet to be determined. Future research may prove that, when it comes to PRP, "the higher the better." Alternatively, it

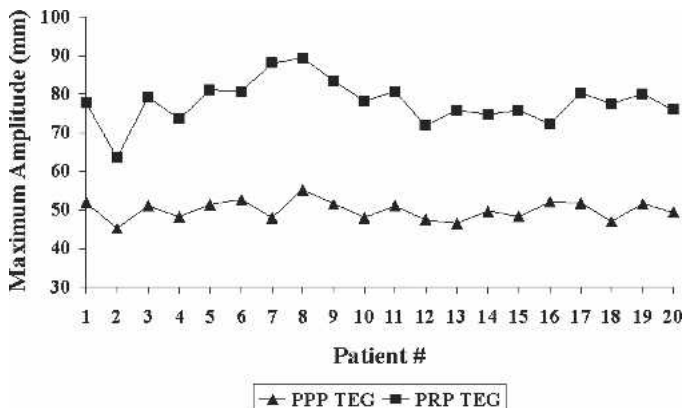


Figure 7. Comparison of the MA for the PPP TEG and the PRP TEG.

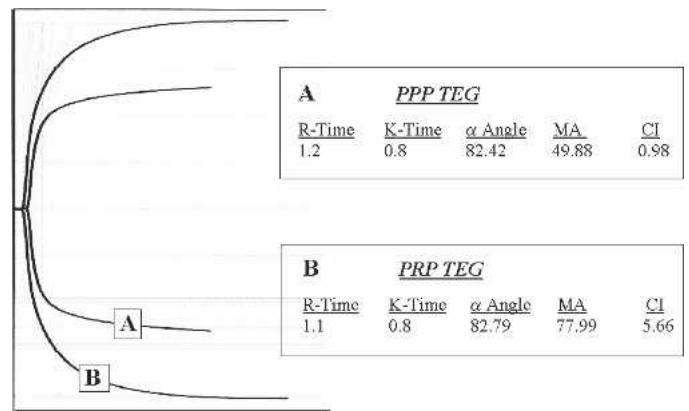


Figure 8. TEG comparison between the PPP TEG and the PRP TEG.

may show that a PRP platelet count of 1,000,000/mm³ is ideal and above that is of no clinical benefit. Perhaps guidelines will be established that will be specific for a particular platelet gel application (plastic surgery, orthopedic surgery, wound care, etc.) rather than a "one size fits all" approach.

This study was conducted exclusively during open-heart surgery. In this environment, harvesting a unit of whole blood (450 mL) pre-bypass for infusion post-bypass is not an uncommon practice (18–22). However, it would be imprudent to use this technique (normo-volemic hemodilution) in settings outside the operating room where patient monitoring may be inadequate (i.e., dental suite, wound care center). We believe that the use of an autologous blood recovery system, such as the CS5, is an acceptable alternative to a dedicated PRP device for open heart surgery. In a setting such as ours, where autologous blood recovery is routinely performed on all cases, the incremental cost (\$50.00) to provide sufficient quantity and quality of PRP is nominal.

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