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Plasmapheresis Techniques During Cardiac Surgery

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Abstract

Plasmapheresis is an aggressive autologous blood conservation method utilized in cardiac surgery to reduce patient exposure to homologous blood transfusion. Presently the three perioperative techniques which have been used clinically incorporate varying methodology in producing platelet rich plasma (PRP). However, no prospective randomized study has been made to concurrently examine the benefits of individual devices.

Fifty-two consenting adult cardiac patients who met selection criteria were randomly assigned to one of three plasmapheresis devices: Plasma Saver (PS), Cell Saver (CS), and Autotrans 1000 (AT 1000). Following induction of anesthesia, 20% of each patient's estimated plasma volume was removed, stored and then reinfused following the reversal of heparin with protamine. One hundred and twenty-two parameters were measured for each patient. These included anthropomorphic, operative, cardiopulmonary bypass, and postoperative follow-up parameters. Indices of hemostasis were measured which included coagulation screens and thrombelastographic data.

There were no differences between groups in all preoperative parameters including the volume of PRP removed. Fibrinogen levels in the PRP were 213.9 +/- 63, 219.4 +/- 73, and 188.9 +/- 69 mg/dl in groups PS, CS, and AT 1000 (p=NS), while platelet counts were 178.4 +/- 73, 121.6 +/- 85, and 210.6 +/- 77 10^9/L, respectively ($p<.05$ CS vs. AT). There were no differences in chest tube drainage, time on ventilator, or length of ICU stay between groups. However, patients in PS group had significantly lower discharge platelet counts than groups CS and AT 1000. Total homologous blood exposure rate (donor blood exposure per patient) was 8.2 units in group PS, 4.5 in CS, and 5.4 in AT 1000, (p=NS).

The currently available techniques for perioperative PRP production differ in both methodology and platelet yield, although the difference did not result in significantly different patient postoperative outcome indices.

Introduction

Transmission of infectious diseases, transfusion reactions, and metabolic abnormalities have always been among the inherent risks of homologous blood transfusions (1). Hepatitis-B, cytomegalovirus and HTLV III are major pathogens whose transmission is associated with increased morbidity, while the risk of contracting acquired immune deficiency syndrome (AIDS) has been estimated from 1:40,000 to 1:1,000,000. (2,3) Clearly, an effort to decrease patient risks associated with this form of therapy led to a search for new methods of autologous blood donation. A successful program for limiting patient exposure should embrace a combination of practices while incorporating perioperative donation. It has been estimated that if all eligible patients had predeposited autologous blood, as much as 72% of their own subsequent transfusion requirements could be met from this source. (1)

Platelet rich plasma (PRP) is defined as a volume of plasma equivalent to 15-30 % of the patient's plasma volume (450-900ml) containing between 1.0-2.5 billion platelets. (4) Platelet pheresis, producing platelet-rich plasma (PRP), is a well established blood bank technique which is suitable in treating

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the majority of cardiac patients who may otherwise not be candidates for perioperative donation. It is also more convenient and less time consuming from a logistical standpoint, both for the patient and the collection facility.

Post-operative bleeding after cardiopulmonary bypass (CPB) commonly results from alterations in platelet number and function. (5,6) Several reports have shown that intraoperative plasmapheresis and post bypass reinfusion of PRP raises the post-operative platelet count (7,8) and reduces the need for blood transfusions. (7,8,9) To date, the vast majority of the reported data has been compiled through the use of one PRP producing device, the Plasma Saver (PS)[a]. However other techniques are also available to generate PRP which include disposable modifications to existing autotransfusion equipment: Haemonetics Cell Saver[a] and Electromedics Autotrans[b]. A prospective randomized study was instituted to determine the efficacy of these devices in generating PRP. Each device utilizes varying centrifuge speeds. The aim of this research is to determine which plasma sequestration technique will be of greatest benefit to the patient.

Materials and Methods

Following approval of the protocol by the institutional Internal Review Board and after obtaining informed consent, patients were randomly assigned to one of the three groups: Plasma Saver (PS)[a], Cell Saver Four with sequestration set (CS)[a], and Autotrans 1000 with sequestration set (AT 1000)[b]. All devices were operated according to manufacturers' recommended procedures. The PS and AT 1000 had similar techniques of direct patient connection, while the CS utilized whole blood gravity draw into a transfer bag with CPD added (CPD to blood ratio of 1:12).

One hundred and twenty-two parameters were measured for each patient. These included the anthropomorphic, operative, and cardiopulmonary bypass results. Post-operative follow-up was monitored from ICU stay to discharge on each patient.

Indices of hemostasis were measured including prothrombin time (PT), activated partial thromboplastin time (PTT) and thrombelastographic data.

All adult cardiac patients routinely operated on at our institution have been evaluated for inclusion in the study. The patient exclusion criteria were:

A. Cardiac patients presenting with critical aortic stenosis (aortic valve area 0.7 cm^2 or less)
B. Left ventricular ejection fraction less than 35%.
C. Left ventricular end diastolic pressure greater than 20 mmHg.
D. Transfusions of blood products 7 days prior to operation.
E. Platelet count less than 150 10^9/L.
F. Pre-op hematocrit (Hct) level less than 35%.
G. Body weight less than 50 kg.
H. Greater than 50% stenosis of the left main coronary artery.

Pre-operative PRP collection was coordinated by the anesthesiologist and perfusionist. The patient's internal jugular vein was cannulated with a 8.5 Fr. catheter for the removal of the patient's whole blood. Prior to initiating plasmapheresis, a pre-skin incision thrombelastograph (TEG) and baseline activated clotting time (ACT) were performed. The patient then underwent plasmapheresis. Hemodynamic status was closely monitored with fluid replacement therapy administered 1:1 with colloidal solutions (6% hetastarch, 5% albumin). Twenty five grams of albumin (SPA) were added to the extracorporeal circuit prime so as to maintain colloid oncotic pressure greater than 11 mmHg for all patients.

Twenty percent of the plasma volume (PPV) was collected from each patient. The following equations were used to calculate this volume.

Estimated patient blood volume

$$(PBV) = kg \times 65\ cc/kg$$

Estimated patient plasma volume

$$(PPV) = PBV \times [1.0 - (Hct/100)]$$

Sequestration volume = 20% X PPV

Once this amount had been collected, the resultant post dilutional Hct was determined, and if acceptable (>20%), the red blood cells were used for reinfusion following CPB. Otherwise, the red blood cells were immediately returned to the patient. The PRP product was sampled for fibrinogen, platelet count, and total protein. The PRP was then stored and agitated at a temperature of 37°C, while the packed red blood cells (PRBC) were stored at room temperature. A ten minute post-skin incision (post PRP removal) TEG and ACT were performed. Following CPB and heparin reversal with protamine, another TEG and ACT were drawn. PRP was reinfused, and after the product was returned, a final TEG and ACT were completed. The remaining CPB blood was processed either by ultrafiltration or centrifugation and was reinfused to the patient with additional protamine given when appropriate.

Coagulation profiles were performed on the first and second post operative days and at discharge, including fibrinogen, platelet count, hematocrit, ionized calcium, PT and PTT. Post-operative chest tube drainage, total input and total output volumes, number of hours on the ventilator, and number of hours in ICU were recorded. Total homologous blood utilization was recorded.

Statistical analysis was performed with a commercial software package[c]. Both one way and two way analysis of

a Haemonetics Inc., Braintree, MA 02184
b Electromedics Inc., Englewood, CO 80155
c Brainpower, Inc., Calabasas, CA

Table 1. Patient demographic data.
BSA = body surface area, pre-op = preoperative.

Parameter	PS	CS	AT 1000	p Value
Age (yrs.)	62.6+/-10.4	63.5+/-9.7	55.2+/-13.7	NS
Sex (male=1)	1.3+/-.5	1.1+/-.3	1.2+/-.4	NS
BSA (m2)	2.0+/-.2	2.0+/-.2	2.0+/-.2	NS
Weight (kg)	84+/-19	90+/-27	86+/-12	NS
Ejection Fraction	55.7+/-14.5	61.3+/-12.7	58.0+/-16.7	NS
Pre-op Hct	40+/-4.0	41+/-4.0	42+/-3	NS

Table 2. Operative data.
CABG = coronary artery bypass graft, IMA = internal mammary artery, CPB = cardiopulmonary bypass. * p=.05.

Parameter	PS	CS	AT 1000	p Value
Operation				
CABG graft #	2.7	3.2	2.7	NS
IMA usage (%)	38	58	65	NS
Valve	2	3	2	NS
CPB Time (min)	121+/-53	126+/-32	97.5+/-36	NS
Cross Clamp Time (min)	70+/29	85+/-20*	63+/-21*	<.05

variance was performed and where statistical significance was found, the multiple comparison (least significant difference) applied. Statistical significance was accepted at the $p < .05$ level. All data appears as mean +/- standard deviation (SD) of the mean.

Results

There were two post-operative deaths. One patient in PS group died on post-operative day two. One patient in AT group died suddenly on post-operative day seven. All other patients recovered and were discharged.

There were no significant differences between the three patient groups in age, sex, body surface area (BSA), and preoperative Hct (Table 1). Surgical procedure, cardiopulmonary bypass time, and cross clamp time are listed in Table 2. Use of the internal mammary artery and cardiopulmonary bypass time were not significantly different between the three groups. Yet, cross clamp time was significantly different between AT 1000 and CS group (63 +/- 21 mins. and 85 +/- 20 mins. $p<.05$).

Table 3 lists the measured values of the PRP product for each group. Platelet counts were significantly different between CS and AT ($p < .05$). Figures 1, 2 and 3 display platelet count throughout patient's hospital stay. The total volume of PRP removed from each group was not significantly different.

Bypass parameters were not different between the three groups. Low CPB HCT was similar in each group at 18.2 +/- 3.1% in PS, 18.0 +/- 2.6% in CS, and 18.1 +/- 1.6% in AT 1000.

Patients' pre-operative coagulation screens (PT, PTT, fibrinogen and bleeding time) showed no difference between the three groups. Although all three groups had a elevation in PT in the immediate post-operative period as compared to the

Table 3. Plasmapheresis Product.
*p =<.05.

Parameter	PS	CS	AT 1000	p Value
Platelet (10⁹/L)	180+/-75	122+/-85*	211+/-77*	<.05
Fibrinogen (mg/dl)	213+/-65	219+/-73	189+/-69	NS
Plasma Volume (ml)	664+/-110	712+/-123	758+/-134	NS
Colloid Oncotic Pressure (mmHg)	13.8+/-1.9	13.5+/-1.8	13.9+/-1.6	NS
Total Protein (mg/dl)	4.8+/-0.6	4.6+/-0.5	4.8+/-0.5	NS
Time (min)	38.8+/-12	36.9+/-16	36.0+/-16.0	NS

Table 4. Postoperative data.
CT drainage = chest tube drainage, POD 1 = post-operative day one, POD 2 = post-operative day two. PRBC Trans. = packed red blood cell transfusion, FFPTrans. = fresh frozen plasma transfusion, Platelet Trans. =platelet transfusion, CRYO Trans. = cryoprecipitate transfusion, HCT =hematocrit.

Parameter	PS	CS	AT 1000	p Value
CT Drainage (ml/24 hours)				
POD 1	870+/-564	1102+/-492	1261+/-960	NS
POD 2	377+/-540	552+/-1071	689+/-1108	NS
PRBC Trans. (units)	2.4+/-2.2	1.6+/-1.9	1.3/-1.7	NS
FFP Trans. (units)	0.8+/-1.5	0.7+/-1.6	0.8+/-1.7	NS
Platelet Trans.(pks)	2.8+/-6.7	1.2+/-3.3	1.5+/-5.0	NS
Cryo Trans. (pks)	2.2+/-5.5	1.2+/-3.2	1.8/-5.3	NS
Discharge Hct (%)	32+/-5	31+/-5	30+/-8	NS
Discharge Platelets (10⁹/L)	196+/-89	243+/-85	253+/-99	<.05
Hospital Stay (days)	10.7+/-2.7	11.3+/-4.2	9.7+/-2.9	NS

preoperative period, only the PS and CS groups showed a significant difference (Figure 4). The PTTs were also prolonged in the immediate post-operative period; and the PS increase reached statistical significance. (Figure 5) Fibrinogen level decreased from the pre-operative to post-operative period, and significant difference was seen for all three groups (Figure 6).

There were no significant differences in chest tube drainage (Table 4), ventilator times, or length of ICU stay between groups.

A significant difference in platelet count at discharge was found between the groups (Table 4). PS had a significant lower platelet count on discharge. Discharge hematocrit levels did not vary between the three groups.

Blood transfusion triggers were set at the following hemoglobin levels: patients 70 years of age and younger, 7 gm/dl and patients greater than 70 years of age, 8 gm/dl. There were

Table 5. Thrombelastograph data.

Pre-skin = pre-skin incision, post-skin = post-skin incision.

Pre-skin	PS	CS	AT 1000	p Value
R Time (min)	8.6+/-3.4	7.0+/-1.0	7.5+/-1.0	NS
K Time (min)	2.9+/-3.1*	1.9+/-1.0	1.7+/-0.6*	<.05
MA (mm)	63.8+/-6.4	61.1+/-17	65.4+/-5.0	NS
MA@ 60 mins. (mm)	57.6+/-7.0	58.3+/-9.7	59+/-5.5	NS
Alpha Angle (degrees)	63.0+/-16.5	64.6+/-10.3	65.9+/-8.6	NS
Post-skin	**PS**	**CS**	**AT 1000**	**p Value**
R Time (min)	7.8+/-1.5	7.2+/-1.8	6.6+/-2	NS
K Time (min)	2.6+/-.9	1.7+/-.6	2.0+/-.9	NS
MA (mm)	57.1+/-7.3*	62.7+/-5.9*	60.2+/-5.0	<.05
MA@ 60 mins. (mm)	49.3+/-6.4	54+/-5.5	53.7+/-5.1	NS
Alpha Angle (degrees)	60+/-7.7	62.3+/-3.4	61.4+/-10.5	NS
10 minutes Post Protamine	**PS**	**CS**	**AT 1000**	**p Value**
R Time (min)	9.2+/-6.5*	6.5+/-1.4*	7.1+/-1.6*	<.05
K Time (min)	4.5+/-2.0*	3.2+/-1.1*	4.4+/-1.6	<.05
MA (mm)	46.4+/-8.2	47.5+/-7.6*	42.0+/-7.6*	<.05
MA@ 60 mins. (mm)	39.6+/-11.2	40.0+/-9.4	35.6+/-11.6	NS
Alpha Angle (degrees)	49.4+/-9.3	51.5+/-8.7	46.9+/-12.1	NS
10 minutes Post PRP	**PS**	**CS**	**AT 1000**	**p Value**
R Time (min)	8.4+/-4.0	7.2+/-1.7	7.4+/-1.7	NS
K Time (min)	4.0+/-1.7	2.9+/-1.1	3.8+/-1.7	NS
MA (mm)	47.6+/-6.5	51.2+/-5.5	47.0+/-6.6	NS
MA@ 60 mins. (mm)	42.0+/-6.2	46.0+/-6.5	42.0+/-6.5	NS
Alpha Angle (degrees)	50.0+/-8.1	54.5+/-9.8	49.1+/-13.6	NS

no significant differences between any group in PRBC, platelet, cryoprecipitate, or fresh frozen plasma transfusions. Although mean homologous blood exposures varied greatly, significance was not met. This can be further illustrated by the number of PRBC and platelet transfusions in each group (Table 4). Total time in hospital was not different (Table 4) between the three groups (p=NS).

There are several parameters which are measured from TEG profile (Table 5) reflecting the kinetics of clot formation and stability. The reaction time (R) correlates with the whole blood clotting time. There was no significant difference noted between the groups during pre-operative period. Post-operative, pre-PRP reinfusion the R time is significantly shorter in CS than PS and AT 1000 (p<.05).

The K time (rate of clot growth) was significantly lengthened in all patients post CPB compared to prebypass values. CS group post protamine was significantly shorter than AT 1000 and PS. The K time decreased in all three groups from 10 minutes post protamine to 10 minutes post PRP (p=NS). The maximum amplitude (MA) (strength of the clot) was increased in all three groups from 10 minutes post protamine to 10 minutes post PRP reinfusion, reaching significant levels in the AT 1000 group (p<.05).

The maximum amplitude at 60 minutes (MA'60) (an indicator of clot retraction and stability) increased in all three groups from 10 minutes post protamine to 10 minutes post PRP reinfusion.

The alpha angle (rate of the clot formation) declined significantly in all three groups following CPB. The alpha angle increased in all three groups following the PRP reinfusion but significant differences were not seen.

Discussion

Intraoperative plasmapheresis offers the cardiac surgical patient a safe and more convenient method of autologous transfusion which may help to avoid homologous blood exposure. Reported benefits include decreased post-operative bleeding, reduced blood bank dependence, and enhanced hemostasis through platelet and factor replenishment. (9,10) Extracorporeal circulation of blood leads to alterations in hemostatic balance, which are associated with induced coagulopathies. (11) Cost is also an important consideration. Fresh frozen plasma is approximately twice as expensive as autologous plasma, and platelets are approximately six times more expensive than the PRP product. (9)

Intraoperative plasma sequestration will remove the platelets undamaged prior to CPB (12). In a post-cardiopulmonary bypass study conducted by Mohr, et al (6) three types of changes in platelets were observed: a decrease in platelet count, a decrease in platelet size, and a disturbance in aggregation response to ristocetin (a platelet aggregator agent). Another explanation for platelet dysfunction after CPB is a disturbance in the prostacyclin-thromboxane equilibrium. (13,14)

There are presently several techniques for plasmapheresis which utilize various commercially available devices. The majority of studies available on plasmapheresis have been completed on one machine with little information available on alternative techniques. (8) The goal of the present study was to examine each of the available clinical techniques currently utilized for perioperative plasmapheresis to determine if clinical outcome could be related to device characteristics.

The Haemonetics Plasma Saver has been utilized for the production of PRP in both the blood bank and the operating room. The system offers variable centrifuge speed to obtain platelet-poor plasma PPP (5,600 RPM) or PRP (3,500 RPM). The donor safety features include ultrasonic air detectors, pressure sensor, plasma collection weigher, peristaltic pumps (blood and anticoagulant), and a flow control system.

Haemonetics Cell Saver with disposable sequestration set includes a three-way distribution line, draw line, and transfer

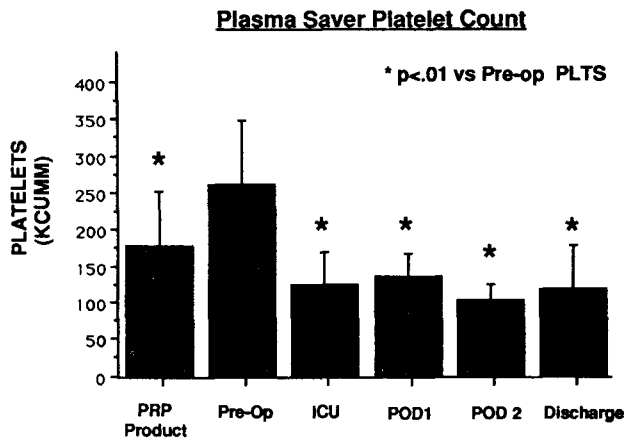

Figure 1.
Platelet Count pre-operation (evening before) and post-operation (drawn immediately upon entry to intensive care unit). * = p<.05 vs. pre-op platelet values within groups. POD 1 = Post-operative day 1. POD 2=Post-operative day 2. Data are means +/- SD.

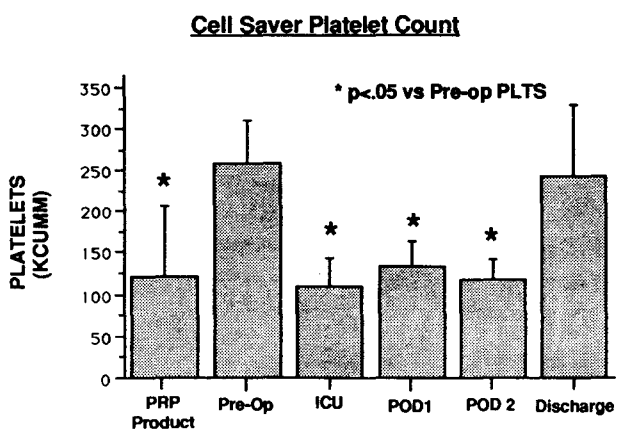

Figure 2.
Platelet Count pre-operation (evening before) and post-operation (drawn immediately upon entry to intensive care unit). * = p<.05 vs. pre-op platelet values within groups. POD 1 = Post-operative day 1. POD 2=Post-operative day 2. Data are means +/- SD.

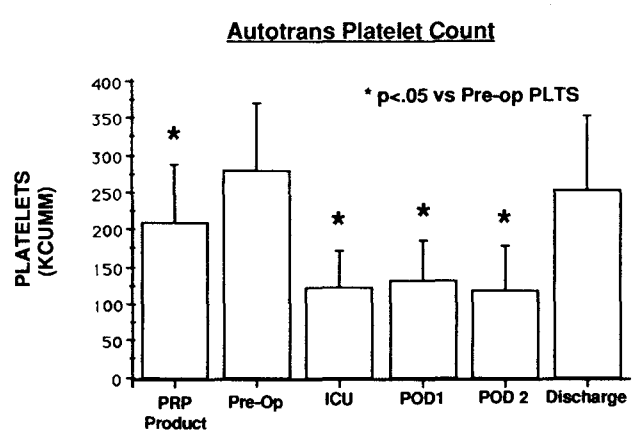

Figure 3.
Platelet Count pre-operation (evening before) and post-operation (drawn immediately upon entry to intensive care unit). * = p<.05 vs. pre-op platelet values within groups. POD 1 = Post-operative day 1. POD 2=Post-operative day 2. Data are means +/- SD.

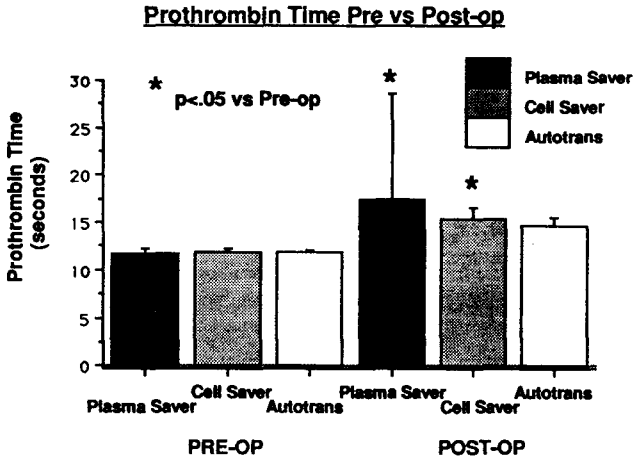

Figure 4.
Prothrombin time pre-operation and post-operation. * = p <.05 vs pre-op. Data are means +/- SD.

pack. Manufacturer recommendations for using the CS include whole blood collection prior to CPB, in separate transfer packs, with separation achieved distant from the patient. The device has a sequestration mode and will fill at a rate of 60-80 ml/min at a centrifuge speed of 4750 RPM.

The Electromedics Autotrans 1000 with disposable sequestration set includes bifurcated patient line with anticoagulant line, burette assembly and semi-vented drip chamber, waste distribution line, and two plasma collection bags. Manufacturer instructions include three separate techniques: a direct patient draw, blood collection bags, and oxygenator venous line withdrawal. The choice was made to utilize direct patient collection, although the manufacturer recommends the

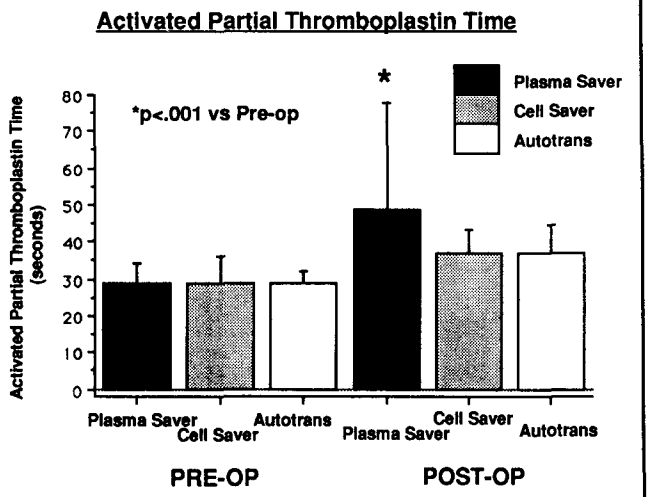

Figure 5.
Activated Partial Thromboplastin time pre-operation and post-operation. *= p <.001 vs pre-op. Data are means +/- SD.

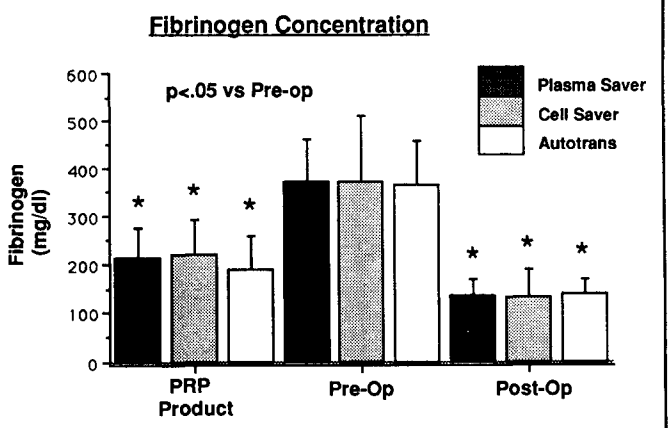

Figure 6.
Fibrinogen concentration pre-operation and post-operation. *= p <.05 vs. pre-op. Data are means +/- SD.

gravity collection method. A software modification to the AT 1000 disables the return mode so that blood cannot be inadvertently pumped back to the patient. Withdrawal flow rate is recommended between 50-75 ml/min, and centrifuge speed is 2400 RPM.

During cardiac surgery there are many causes of coagulopathies. These include inadequate surgical hemostasis, hypothermia, dilution of coagulation factors, inadequate reversal of heparin, the possible effect of excess protamine, reduction in the number of platelets, abnormal function of the remaining platelets, and the activation of various mediator systems due to contact with foreign materials. (15)

CPB leads to acute thrombocytopenia which can be attributed to hemodilution and adherence of platelets to foreign surfaces. (1,8) During CPB platelets are sequestered in the spleen and liver and are not returned to the circulation until some time after bypass. (16,17) Younger platelets are preferentially pooled in the spleen during CPB and are not readily available. (18) Therefore, by sequestering the platelets prior to CPB, and reinfusing them immediately post CPB, they should be readily available for hemostasis.

The advantage of plasmapheresis in comparison to whole blood draw is that red cells can be returned at any time that the hemodynamic condition of the patient necessitates. PRP with all its clotting factors, is reinfused after reversal of heparin with protamine. Heparin is not the anticoagulant of choice for PRP sequestration. Heparin and its neutralizing drug, protamine, may activate platelets and the complement cascade. (19,20) Heparin does not have American Association of Blood Banks approval for blood storage. (21) CPD is approved for blood storage and is used in blood banks for storage of blood products. Through the process of the citrate ion chelating calcium in the blood, CPD makes calcium unavailable to the coagulation system. CPD through the binding of calcium also protects the platelets.

TEG results shown an decrease in K time and increase in MA, MA'60, and alpha angle from post protamine to ten minutes post PRP reinfusion. The AT 1000 reached the significant level in the MA and MA'60 parameters. Wong et al, used the TEG and found no increase in MA or improvement in alpha angle immediate post PRP. (22) Our noted improvement is probably due to waiting ten minutes before a TEG was drawn.

Giordano, et al have looked at the effect of the use of autologous PRP in several multicenter trials. Their results showed a significant decrease in homologous plasma and platelet usage when autologous PRP is used in cardiac surgery. (9,16,23)

In a separate study Jones, et al have shown that 32 % of the control patients and 66% of the plasmapheresis patients were sent home without requiring homologous blood transfusions ($p<0.001$). (24) A volume of 600 to 800 ml of PRP should be adequate to correct the plasma clotting protein deficit stimulated by cardiac surgery with CPB. (25)

The results of our study show that the AT 1000 system had the greatest platelet yield in the PRP product. This is most likely a result of centrifuge forces involved in the separation of substances of varying densities. During centrifugation the heavier components of blood will be located at a point most distant to the axis of rotation. Newly formed platelets are large and heavy. (26) This perpendicular layering could cause the younger, healthier platelets to be located in strata below lighter

particles and, hence, separated by the continuous flow of blood into the centrifuge. The three plasmapheresis machines all had different levels of centrifugation which result in varying gravitational force. The AT 1000 in the plasmapheresis mode operates at 2400 RPM, while the PS and CS are preset at 3500 and 4750, respectively. The lower G force of the AT 1000 causes a less dense packing of the cellular elements of blood. The platelets, therefore, are less likely to be withheld in the layering effect of centrifugation and this may result in the significantly elevated platelet yields seen in that group. Another possible explanation may be that the AT 1000 group sequestered additional buffy coat layers because of the less dense packing. This would increase platelet yield. Although we did not measure the quality of buffy coat layers obtained in each group, we consistently collected only 20% of patient predicted plasma volume. Therefore, since patient preoperative Hct did not vary, each group had the same chance of capturing a second buffy coat layer.

Another concern on PRP collection is the geometry of the bowl used for pheresis. Both the CS and AT 1000 use the standard 225 ml Latham bowl for centrifugation. The PS, however, has a specially designed conical bowl especially suited for platelet pheresis. We were unable to show a difference between groups according to bowl characteristics.

The present study was designed as a comparison between three commercially available devices used for intra-operative plasmapheresis. The results of previous studies have established the efficacy of this treatment in reducing patient exposure to homologous blood products. The major differences between the devices were in bowl geometry and varying centrifuge speeds. Although the Autotrans 1000 generated the greatest platelet yield, a clinically significant benefit was not seen.

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