Lecture

Platelet Rich Plasma (PRP) Sequestration Why, When, How?

Paul A. Page, B.S., C.P., P.A.
Electromedics, Inc.

Keywords: platelet rich plasma sequestration, PRP, plasmapheresis, autologous, plasma sequestration, plasma saving system, platelet transfusion

Abstract

Reduction in the amount of blood products transfused during and following any surgery is vitally important because of the risk of blood-transmitted diseases and the increasing costs associated with any transfusion. During the 1970s, a technique of sequestering (drawing off and storing) a patient's blood in the operating room evolved in cardiac surgery. This procedure involved the removal of one or two units of whole blood into anticoagulated storage bags prior to going on cardiopulmonary bypass. This autologous blood was stored in the operating room at room temperature until the patient was brought off cardiopulmonary bypass and stabilized. The blood was then reinfused into the patient. It was theorized that the blood withdrawn contained fresh platelets, red cells, and coagulation factors required to help restore hemostasis rapidly. Furthermore, oxygen carrying capacity following the operation was improved, thereby reducing the need for homologous blood product transfusions following the operation.

An entirely new approach to reduction in the use of blood products involves the preoperative collection of Platelet Rich Plasma (PRP) and the subsequent reinfusion of the PRP containing fresh, undamaged platelets and clotting factors following the operation.

This paper will document why the technique of utilizing Platelet Rich Plasma Sequestration is beneficial for the patient, when and where platelet rich plasma sequestration can be performed and, how the PRP procedure is technically performed.

Why Perform PRP?

The recent awareness of transfusion-related diseases has made the reduction of homologous blood or blood product transfusions highly desirable. A current article by Lavee J, et al. illustrates that the technique of sequestering whole blood is still being utilized effectively.[1] This article reported on a study that evaluated the effect of fresh whole blood transfusion versus the transfusion of blood bank platelet concentrates on platelet aggregation after cardiac operations. The results demonstrated that one unit of fresh whole blood restored platelet aggregation on the extracellular matrix to a preoperative status. In comparison, eight units of stored blood bank platelets were needed to achieve the same results. One unit of fresh whole blood increased platelet count in a manner similar to that achieved by six stored platelet units from the blood bank. In addition, the whole blood increased the mean platelet volume to a level higher than that achieved by administering 10 banked platelet units. These results seem to suggest that the effect of one unit of fresh whole blood (containing fresh platelets), on platelet aggregation after cardiopulmonary bypass is at least equal, if not superior, to the effect of eight to ten stored platelet units from a blood bank. It is important to note that the whole blood utilized in this study would have fresh platelets as opposed to the stored blood bank platelets.

Historically, platelet collection was performed by the blood bank one to five days in advance of surgery. When blood bank platelets were ordered for a patient during surgery, the platelets were, on the average, three days old. An entirely new approach to aid in the reduction of the use of homologous blood products involves the preoperative collection of Platelet Rich Plasma (PRP) and the subsequent reinfusion of these fresh, undamaged platelets and clotting factors following the operation. Several studies have demonstrated that preoperative withdrawal and storage of autologous plasma (fresh frozen plasma) is effective in blood conservation for patients undergoing elective procedures in orthopaedic surgery, neurosurgery, open heart surgery, and other elective operations. Engelman, for example, reported that the use of this technique resulted in significantly decreased postoperative hemorrhage in coronary bypass patients.[2]

With current techniques of autotransfusion (cell washing), only the washed red cells are concentrated and returned to the patient. The plasma, containing proteins, platelets, clotting factors, etc., is washed from the red cells and discarded. In operations where patients have a large amount of blood loss and autotransfusion is utilized, a substitute for this lost plasma volume must be administered. In addition to electrolyte and

Address correspondences to: Paul Page, Marketing & Technical Support Manager, Electromedics, Inc., 7337 S. Revere Parkway, Engelwood, CO 80112

colloidal solutions, homologous and/or autologous fresh frozen plasma (FFP) can be given. Von Finck, et al. studied the effects of different substitutes on the clotting reaction in a group of patients undergoing extensive orthopaedic and traumatologic surgical operations.[3] He reported that when an operation can be planned in advance, the necessary autologous FFP can be obtained from the patient. As a result of utilizing autologous FFP, the operative and postoperative blood losses are reduced. The results of this study demonstrate that it is possible, for example, in total hip prosthesis replacements, for 90% of all cases to be done without using homologous blood or plasma.

Ron Nichols, in his paper Sequestration Advantage, reported a dry post-pump patient who survives with minimal support and is transfused with little, if any, bank blood products, is the major objective of most perfusionists today.[4] He also reports that the pre-bypass blood sequestration program, which he has used for several years, has proven itself to be a very valuable asset in reducing red cell transfusions by providing the anesthesiologist with an alternative to banked blood for immediate post-bypass volume replacement. One of the advantages he reported in using the cell washing system to perform the platelet rich plasma sequestration in the immediate preoperative period, was that the red cells could be returned to the circuit sooner, possibly limiting the amount of crystalloid solutions required for the case. This advantage, while not enough to prevent banked platelet infusions, may result in a much drier post-op course.

Jones, et al. reported on the effects of intraoperative plasmapheresis on blood loss and homologous transfusion in cardiac surgery in 100 patients.[5] The results showed that the group receiving intraoperative plasmapheresis had a significant reduction in operative red cell mass lost, a reduction in the average homologous transfusion and an increase in the percentage of patients not requiring any blood transfusions. Giordano et al. also studied the use of autologous platelet rich plasma (PRP) in Cardiac Operations.[6] Their results also demonstrated a decrease in homologous blood use and bleeding when autologous platelet rich plasma was collected in addition to the use of intraoperative autotransfusion.

When and where is PRP performed?

In the past, platelet collection was performed by the blood bank one to five days in advance of scheduled surgery. Plasma collection, depending upon the need, was normally performed several times preoperatively. In a subject of normal weight and height, about 900 cc of plasma would be collected each time. The collection of autologous plasma was usually completed by the blood bank at least 14 days before surgery.[7]

With the advent of autotransfusion devices that feature variable speed centrifugal cell separators, the ability to separate the red cells and other blood components became available in the operating room.

Platelet Rich Plasma (PRP) sequestration is a technique in which blood is removed from a patient, transported directly into a centrifuge bowl, and separated into platelet rich plasma and packed red cells. Once the blood has been separated, the individual components can then be stored in the operating room and/or reinfused as required.

There are two techniques for withdrawing blood for plasma sequestration in the operating room during the immediate preoperative period.

The first technique utilizes a direct patient connection. This technique does have several drawbacks. It requires the use of isolated anticoagulation for the blood being withdrawn and processed. This technique requires the use of a special patient drawing line that incorporates a Y connector at the patient drawing site. This allows anticoagulant solution to be mixed with the blood as it is withdrawn from the patient. In addition, the patient must be continuously monitored and replacement fluids infused during the entire blood withdrawal period. Blood withdrawal flow rates are usually in the range of 50-75 cc/min.

With the second technique, the blood is withdrawn from the patient into blood transfer bags containing an adequate amount of CPD anticoagulant solution. This is usually done by the anesthesiologist. Since the anesthesiologist is monitoring the patient, controlling the blood withdrawal rate and the replacement fluid flow rates, the collection process is somewhat easier than the direct patient connection technique.

von Bormann[7] believes that ideally the plasmapheresis procedure should be done after premedication but before onset of anesthesia. Others, however, have found it more convenient to perform the procedure after the induction of anesthesia.[8] There is also some debate as to whether to perform the blood withdrawal prior to systemic heparinization of the patient (in cardiac surgery). Sometimes in cardiac cases there are not sufficient personnel available to perform direct patient connection and withdrawal, or there is not enough time for the anesthesiologist to perform withdrawal in CPD transfer bags. In these instances clinicians, like Ron Nichols[4], have collected blood for PRP after systemic heparinization. Collection of the blood into CPD transfer bags is accomplished immediately after the clearing of the prime solution from the venous perfusion line, but before the onset of bypass. This technique is gaining in popularity for open heart surgery cases. The procedure is carried out using a Y connector with two fast prime 1- inch lines going into two CPD transfer bags (see Figure 1).

This adapter is placed in the venous line of the cardiopulmonary bypass pump (see Figure 2). This allows the two plasma sequestration transfer bags to be filled in 30-40 seconds immediately prior to the initiation of total cardiopulmonary bypass. When using this technique caution must be exercised to ensure that the $^{1}/_{4}$-inch arm of the Y on the venous access site is securely clamped with a tubing clamp. In addition the operator must close the duraclamps on each of the collection arms of the adapter. This will assure that no air can enter the venous line of the pump system from the sequestration adapter. It is important to note that if the blood collection is performed, even after systemic heparinization, CPD

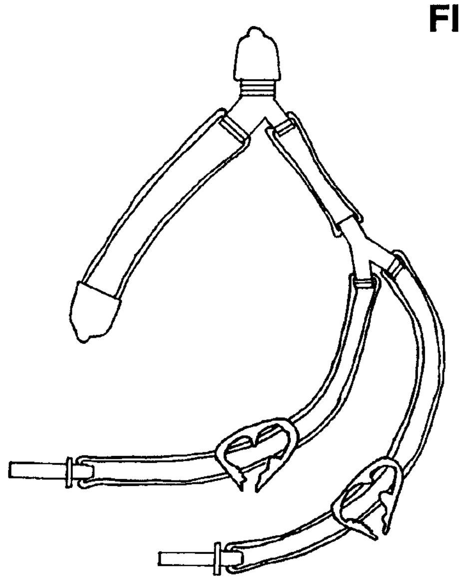

FIG. 1

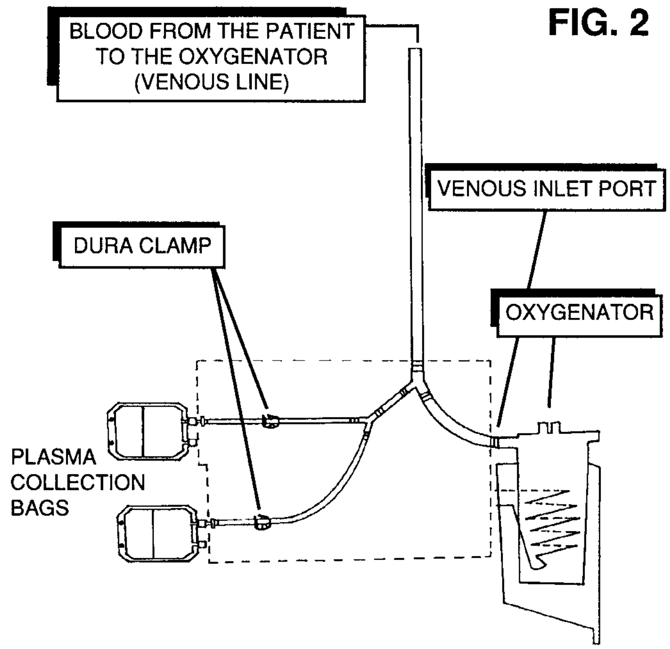

FIG. 2

anticoagulant solution still should be added to the collection transfer bags prior to the collection of the blood. The addition of the CPD is required because CPD anticoagulates the blood utilizing a different mechanism than heparin, thereby affording more protection for the platelets during the storage and separation processes.

How is PRP performed?

Outlined below are the basic equipment, supplies and procedures required to perform PRP sequestration.

Autologous platelet rich plasma sequestration is defined as:

A volume of plasma equivalent to 15-30 percent of the patient's plasma volume (450 - 900 ml) containing 1.0 - 2.5 X 10^{11} platelets

In the normal patient, approximately 20-25 percent of the patient's circulating plasma volume should be the collection goal for efficacious PRP. Consequently, the initial step in the sequestration process is to calculate how much plasma must be collected. To calculate the plasma volume the estimated blood volume of the patient must first be calculated (see Table I). Once the blood volume has been determined the total plasma volume may be calculated. The formula for calculating the total plasma volume is shown in Table I, and an example of how to calculate the plasma volume is shown in Table II. The final step is to calculate the amount of plasma to be sequestered. Once the desired percentage of the patient's total plasma volume to be sequestered is decided upon, the actual amount of plasma to be sequestered can be calculated as shown in Table III.

Table I
Steps to Calculating Plasma Volume

To estimate the plasma volume of a patient follow these two steps:

Step 1. - Calculate the estimated **B**lood **V**olume
BV_c = Kg X 70.0 cc/Kg

Step 2. - Calculate estimated **P**lasma **V**olume
PV_c = (BVc) X [1.0 - (Hct/100)]

Table II
Calculation of Plasma Volume

Patient Hct = 48%
Patient BVc = 5,250 cc
PV_c = 5,250 [1.0 - (48/100)]
PV_c = 5,250 X [1.0 - (0.48)]
PV_c = 5,250 X 0.52
PV_c = 2,730 cc

Table III
Determination of the Estimated Sequestration volume

SV_c = PVc X % extracted
SV_c = 2,730 cc X 25%
SV_c = 682.5 cc

Von Bormann[7], in discussing the hemodynamics of donor plasmapheresis, offers a simplified calculation method. He suggests collecting 10 cc plasma/kgbw. In the above example this would equal 700 cc which is only a difference of 17 cc of plasma.

There are many plasmapheresis machines on the market at this time that perform PRP. However, only a few of these units have been approved by the FDA for use in the operating room or patient care areas. These units were initially designed to be utilized in the blood bank or outpatient departments.

The techniques described in this paper pertain to some autotransfusion machines which, with the right disposables and training, allow PRP sequestration to be performed by a perfusionist or autotransfusionist in locations outside the blood bank, such as the operating room, preoperative holding area, or even in the patient's room.[9]

The following supplies will be needed to perform PRP:

- Centrifugal separation type autotransfusion machine with variable speed centrifuge that can be adapted to perform PRP.
- Appropriate centrifugal bowl and tubing kit.
- Appropriate Plasma Sequestration Disposables Kit.

Sequestration Procedures:

1. Install the PRP disposable accessories on the autotransfusion unit according to the manufacturer's instructions.
2. Blood Collection.

a. If performing sequestration directly from the patient, set up and connect blood withdrawal line to the patient. NOTE: The fluids selected for volume replacement must be selected and/or approved by an attending physician (typical fluid replacement solutions are lactated Ringers solution, normal saline, saline with dextrose, normosol, plasmalyte, any other balanced electrolyte solution, albumin or other selected colloidal solution).

b. If performing sequestration directly from blood stored in blood transfer bags, withdraw blood from the patient into blood collection bag(s) that have been properly anticoagulated with selected CPD anticoagulant solution as recommended by the AABB or hospital guidelines (usually 15 cc of CPD for each 100 cc of blood collected).

3. Before starting blood collection for PRP, override the autotransfusion unit's red cell level detector (if the machine is equipped with one) so the bowl can be completely filled with red cells. The required centrifuge speed setting for optimum PRP collection is 2,400 RPM. Set the centrifuge speed prior to the start of blood collection. Very slowly (50-75 cc/min) withdraw the blood, mixed with the appropriate amount of anticoagulant, from the patient or the blood transfer bags, filling the centrifuge bowl completely with red cells, while collecting the plasma into plasma storage bag(s). Care must be exercised when performing plasma sequestration. A 225 cc centrifuge bowl can yield 800 cc or more of plasma, causing hypovolemia if the patient's fluid balance is not carefully maintained. Many variables influence the amount of plasma that can be sequestered. The determination of the volume to be sequestered and the fluids selected for volume replacement must be calculated and/or approved by an attending physician. In addition, advise the physician of the amount and type of anticoagulant utilized, since the plasma collected will still contain residual anticoagulant solution. A normal sequestration procedure consists of sequestering blood from two bags of blood (or 900-1000 cc of whole blood withdrawn directly from the patient). This would normally yield 400-800 cc of platelet rich plasma, depending on the patient's hematocrit.
4. When the required amount of plasma has been collected, red cells left in the centrifuge bowl may be transferred to the blood holding bag.
5. Transfer processed red cells from the holding bag to a blood transfer bag. When desired, administer red cells back to the patient through an IV transfusion set using an appropriate blood filter.
6. Retain the platelet-rich plasma for reinfusion upon the physician's order. Utilize procedures consistent with your blood bank's protocol for platelet product storage and handling.
7. The above steps may be repeated until the required amount of PRP has been collected, based on the previous blood and plasma volume calculations.
8. Platelets prepared as described should be stored at 20-24°C with continuous gentle agitation. Plateletpheresis concentrates that have been prepared in an open type system such as this, may be stored for no longer than 24 hours.[10] Hutchison reported that post-transfusion survival values were better in platelets that had been stored at 22°C if they were incubated at 37°C for 1 hour prior to transfusion, than platelets maintained at 22°C up to the time of transfusion.[11]
9. Once the sequestration procedure is completed, the autotransfusion unit can be converted to normal autotransfusion operation, which will provide additional blood conservation opportunities for the patient as well as cost savings.

Conclusion

The sequestration and re-administration of whole blood has been reported to be successful in reducing bleeding and reestablishing hemostasis following open heart surgery cases for the past 20 years. Platelet concentrates and fresh frozen plasma have been used for years by anesthesiologists and surgeons following major surgical procedures to promote hemostasis and restore clotting factors and platelets to physiologic levels.

The drawback in the past has been that, for the most part, these were administered as homologous blood products. In view of recent increased awareness of the risk of blood-transmitted diseases and the increasing costs associated with any transfusion, a reduction in the amount of blood products transfused during and following any surgical procedure is vitally important. The techniques outlined in this paper for autologously sequestering a patient's plasma and platelets in the operating room area for later reinfusion provide a method for the surgical team to help reduce to a minimum the amount of transfusions required by the patient, and possibly even eliminate the need for any homologous blood/blood product transfusions.

Bibliography

1. Lavee J, Martinowitz U, Mohr R, Goor DA, Golan M, Langsam J, Malik Z, Savion N. The Effect of Transfusion of Fresh Whole Blood Versus Platelet Concentrates After Cardiac Operations. J Thorac Cardiovascular Surgery 1989; 97:204-212.
2. Engelman RM. Blood Conservation During Open-Heart Surgery. Proc Asian Congress on Thorac & CV Sur 1989; 9:16-16.
3. von Finck M, Eulert J, Heller W, Sohorer R. Autotransfusion and plasmapheresis in preparation for surgery: Behavior of coagulation. Anaesthesist 1985; 34:675-680.
4. Nichols RD. Sequestration Advantage. Perfusion Quarterly 1989; 5(1):1-4.
5. Jones JW, McCoy TA, Rawitscher RA, Lindsley DA. Effect of Intraoperative Plasmapheresis on Blood Loss and Homologous Transfusion in Cardiac Surgery. The Annals of Thoracic Surgery 1990; 49(4) April:585-590.
6. Giordano GF, Giordano Jr GF, Rivers SL, Chung GKT, Mammana RB, Marco JD, Raczkowski AR, Sabbagh A, Sanderson RG, Strug BS. Determinants of Homologous Blood Usage Utilizing Autologous Platelet-Rich Plasma in Cardiac Operations. Ann Thorac Surg 1989; 47:897-902.
7. von Bormann B, Boldt J, Schleinzer W, Kling D, Hempelmann G. Hemodynamics in Donor Plasmapheresis. Anaesthetist 1989; 37:316-320.
8. Vertrees RA, DelRossi AJ, Cernaianu AC, Cilley JH, Baldino WA, Wacker C, Fuller SJ. Does Plasma Sequestration Reduce Postoperative Bleeding? JECT PROCEEDINGS 1989; 27 Int Conference:104-llO.
9. Page, PA. Sequestration. Electromedics Blood Conservation UpDate 1990; 4(1):3-5.
10. AABB Technical Manual l0th Edition, 1990. Richard H Walker, Editor. pp 23
11. Hutchinson, KD Kunkel, MJ Schell, CW Jackson, EJ Nelson, WC Wang, SJ Fischl, LB Taylor, CH Pui: Brief 37°C Incubation of Platelets Improves Response to Transfusion. Transfusion, 28:S-9, 1988.