Platelet and Granulocyte Pheresis

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ABSTRACT

The need for sophisticated component therapy has resulted in improved
techniques for obtaining concentrates of platelets and granulocytes. The use
of single donors as a source for these products is advisable to avoid multiple
sensitizations. A method for obtaining large quantities of platelets has been
established rather easily. Obtaining concentrated granulocytes represents a
greater problem owing to the difficulty in separating granulocytes from red
cells by differential centrifugation or sedimentation since the specific
gravities are similar. Separation is easier by the use of hydroxyethyl starch. A
modified solution of hydroxyethyl starch and sodium citrate was used with
good results.

Introduction

The increasing demand for specific blood components such as platelets and
granulocytes, has resulted in many advances in hemotherapy. Vigorous chemo­
therapy, as applied in the treatment of leukemias, lymphomas and other
neoplasms, produces bone marrow depression almost universally. In addition,
adverse reactions to commonly used drugs and medications have required
supportive therapy in these conditions. Aplastic anemia, neutropenia, drug-
induced thrombopenia and even massive transfusions, which "wash-out" platelets
and the more labile coagulation factors, are seen more frequently owing to aggressive
modern treatment of disease.

How to satisfy the need for the large quantities of these specific blood particu­
late components has occupied the attention of hematologists and blood bank per­
going for several years. The results of their research have been the production of
several mechanical devices for the concentration of platelets and granulocytes
from blood without adverse effects on the donor and with proven viability and effec­
tiveness of the final product. Discontinu­ous and continuous centrifugation, nylon
filtration and differential sedimentation have all been utilized. Each technique
has shown advantages and disadvantages to a greater or lesser degree.

In our hands the cell separator* has demonstrated the ability to produce a
good final product with minimal donor risk and discomfort. The recipients
have shown effective therapeutic results, judging by correction of the initial defects.

* Haemonetics #30 Cell Separator, Haemonetics Corp., Natick, MA.
The technique for the use of the equipment is easily learned. The advantage of a single donor source who can be repeatedly pheresed, thus reducing the risk of sensitization and disease transmission, makes this equipment particularly desirable. Of most importance is the absence of risk or danger to the donor.

Donor Selection

Although still somewhat controversial, the use of type and Rh specific donors seems advisable since some red cell debris or even intact cells may be present in the final product. In addition, there is evidence that both platelets and granulocytes share antigens with the red cells; therefore, incompatibility between the donor and the recipient should be avoided. HL-A specific donors would be ideal; however, this is rarely possible or practical. In most situations, it is advisable to use siblings or close relations of the patient in the hope of at least a haplotype or closely matched donor. Males and females can be used. Donors should not have taken aspirin-containing medications for the previous five days. Moderate anemia is not a contraindication since the red cells are returned with minimal loss to the donor. The first cycle removes 500 to 700 ml of blood from the donor. Therefore, it is advisable (1) to have the donor in a Trendelenberg position, (2) to use adequate fluid replacement at the beginning of the procedure and (3) to perform the first cycle at a slightly lower rate than the following cycles. This permits the donor to adjust to the volume changes without discomfort. The donor should be kept warm (blanket if necessary) since the reinfused blood is cooled by the ambient room temperature.

Compatibility tests between the donor and recipient should be carefully performed and the American Association of Blood Banks (AABB) requirements for the avoidance of disease transmission followed. This means the history should be taken carefully to rule out malaria, hepatitis, viral disease, recent dental extractions or operations. The donor’s serum should be tested for syphilis and HbsAg. Ample time should be taken to motivate the donor by a discussion of the benefit his contribution can provide, by an explanation of the mechanics of the procedure and by reassurance as to the harmlessness of the pheresis. Since repeat pheresis from the same donor is nearly always needed, this time is well spent.

Additional Hints

A clean, brisk venapuncture is essential. Besides making the procedure easier, a proper venapuncture improves the donor’s willingness to undergo further pheresis. The use of the Trendelenberg position eliminates syncopal reactions. The rate of the phlebotomy should be slow for the first cycle to avoid faintness. As the bowl fills to the outflow tract, the speed should not exceed 15 to 20 ml per min to avoid turbulence which can lower the yield.

Although six cycles are usually done, this number can be increased to eight or even 10 if the donor is a large, experienced donor. Leukopheresis can be repeated daily, if necessary, since the granulocyte pool is very large (95 percent of the granulocytes are extravascular and “on call”). Since the half-life of the granulocyte in the circulation is measured in hours, it may be necessary to supply granulocytes this often. Platelets have a half-life of several days and, therefore, require repeat infusion less frequently. In addition, there is a moderate drop in the number of platelets in the donor following plateletpheresis. A recovery period of a day or two is thus necessary.

Procedures

Platelet Recovery

The procedure followed depends upon the particular end product being sought.
Thus, for platelet recovery, the anticoagulant consists of 2 to 3 percent trisodium citrate in distilled water. The sodium citrate can be obtained in 30 ml vial containing 17 g of trisodium citrate*.

Approximately 25 ml of this concentrate are added to 500 ml of distilled water (for intravenous use). With the lines flushed with the anticoagulant, a clean venapuncture is made in the largest antecubital vein present, with a blood pressure cuff inflated to approximately 40 mm of pressure. The machine is then turned on by pressing the “Fill” button, and the volume control turned up so that 20 to 40 ml of blood is collected per min, as judged from the indicator meter. The monitor pouch is carefully observed. In the event that the pouch collapses, the donor is instructed to slowly open and close his hand, and the volume control lowered. This is repeated as necessary throughout the procedure, with whatever adjustment in the position or angle of the phlebotomy needle is found to give the best yield.

Again, the first cycle is drawn slower than those following in order to preclude any fall in blood pressure which would decrease the donor’s blood flow. As the bowl fills, the clear separation between the plasma and the red cells can be seen with the thin white line of platelets between. The plasma bag will be filling, and the platelet line will rise to the outflow port. When the rapidly broadening platelet line reaches toward the outflow port (with about 0.5 to 1 cm of plasma still present), the next button (marked platelets) is pressed. This directs the flow of platelets to bag No. 1. The speed of the flow from the donor during this time should be reduced to 10 to 15 ml per min to reduce turbulence. The line to the platelet bag is carefully observed. As soon as the pink color of the red cell layer is seen, the next button is pressed which directs the flow to bag No. 2 white blood cells (WBC). This bag is kept on a gram scale.* The blood flow to this bag is continued until 30 g (roughly 30 ml) has been collected.

At this point, the processor is stopped (by pressing the stop button), and the blood pressure cuff released. After the bowl has come to a standstill, the next button (button marked empty) is pressed and the flow regulator slowly turned up toward the maximum. This pumps the plasma and the residual red cells to the overhead reinfusion bag. A slow saline drip is then started through the phlebotomy, and a small amount of the saline solution is flushed through the system to avoid any stasis.

When the reinfusion bag contains about 50 ml, the return clamp to the donor’s opposite arm is opened and the reinfusion begun. The saline to this needle is shut off during the reinfusion. The bowl and plasma bag will soon be emptied, and this is noticed as the bowl empties or as an air bubble appears in the line to the reinfusion bag. The pump is then stopped, the gauge returned to zero and preparation made for the second cycle, while the blood is still being reinfused to the donor. The blood pressure cuff is reapplied, the saline reinfusion is stopped and the centrifuge again begun by depressing the fill button, and gradually increasing the speed of the phlebotomy by turning the regulator.

When only a few ml of blood remain in the reinfusion bag, the saline drip to the reinfusion arm is restarted (drops!) and the clamp on the line from the reinfusion bag tightened.

This procedure is repeated for the number of desired cycles (usually six to seven) with each cycle taking about 15 to 20 min. In less than two hours, there will be collected approximately 100 ml in the platelet bag and 200 ml in bag No. 2 which contains platelets, (WBC) (mainly lymphocytes) and red blood cells (RBC). While the last reinfusion is taking place,

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* McGraw Laboratories, Irvine, CA.

* Hanson Dietetic Scale, Northbrook, IL.
TABLE I
Harvesting of Platelets

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Yield</th>
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<tbody>
<tr>
<td>500 ml of sterile H₂O + 15 g of tri-sodium citrate</td>
<td>4 x 10¹¹ (average)</td>
</tr>
<tr>
<td>Bag #1 (21 donors) = 1 x 10¹¹ per L (average)</td>
<td></td>
</tr>
<tr>
<td>Bag #1 + #2 (90 donors) = 1.2 x 10¹¹ per L (average)</td>
<td></td>
</tr>
<tr>
<td>Bag #1 + #2 + residue (42 donors) = 1.5 x 10¹¹ per L (average)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 ml of 6 percent hydroxyethyl starch (HES) + 15 g of tri-sodium citrate</td>
<td>2.8 x 10¹¹ (average)</td>
</tr>
<tr>
<td>Bag #1 (41 donors) = 0.7 x 10¹¹ per L (average)</td>
<td></td>
</tr>
<tr>
<td>Bag #1 + #2 (19 donors) = 1.5 x 10¹¹ per L (average)</td>
<td></td>
</tr>
<tr>
<td>Bag #1 + #2 + residue (10 donors) = 1.95 x 10¹¹ per L (average)</td>
<td></td>
</tr>
</tbody>
</table>

Average yields with and without hydroxyethyl starch (HES) using tri-sodium citrate as anticoagulant.

Bag No. 2 can be removed to a refrigerated centrifuge and spun at 800 rpm for seven min at 22°. The supernatent platelet rich plasma can then be added to the platelet bag, and the residual RBC (usually 70 ml) can be returned to the donor. As will be seen in table I, approximately 33 percent of the shed platelets are still in the RBC sediment. If the slight loss of RBC is not consequent to the donor and the compatibility tests have been done, the entire contents of the plasma bags #1 and #2 can be combined and used for the patient. (After thorough mixing of the combined bags, a segment is taken for counting of platelets, WBC, etc., and the bag weighed to calculate the total number of platelets obtained).

GRANULOCYTE HARVESTING

For granulocyte harvesting, a different technique is necessary. To obtain a satisfactory yield the donor is given an i.v. injection of dexamethasone (6 mg) about two to three hours before starting the procedure or 12 mg orally 10 hours prior. This produces a leukocytosis and granulocytosis in the donor which is maximum in about three hours, if given i.v. or 8 to 10 hrs if given orally, at which time the phlebotomy is begun. The anticoagulant (15 g of tri-sodium citrate obtained from a 30 g vial described previously) is added to 500 ml of hydroxyethyl starch* and the procedure follows the same pattern as for collecting platelets.

Upon completing the phlebotomy of six to eight cycles, bag #2 (which contains WBC, RBC and platelets) is allowed to hang from a support and owing to the hydroxyethyl starch in the bag, sedimentation takes place rapidly. In 15 to 20 min, the RBC and plasma containing WBC and platelets are clearly separated. The WBC-rich supernatent plasma is then taken off to a satellite bag and the residual RBC returned to the donor. The greatest yield of WBC and platelets can be obtained by transfusing the donor with the entire yield (in bag #2 of WBC, RBC and platelets); however, the necessity of repeatedly using the donor for several days would involve too great a loss of RBC. Therefore, if the donor is to be used daily, as may be needed, the RBC's should be returned. Yields obtained by this technique are shown in table II.

The effect of hydroxyethyl starch on the donor is a temporary increase in blood volume (plasma expander effect of hydroxyethyl starch). This rapidly returns to normal with no harmful effects being reported.

Suggested Techniques

1. The donor should be in the Trendelenberg position.
2. A clean venapuncture is essential.
3. The first cycle should be done slowly so as to avoid hypotension and syncope. The saline infusion for this first cycle should be rather rapid (equal replacement?).
4. The procedure can be continued by drawing 20 to 60 ml per minute; however, as the bowl fills, the speed should be readjusted to 10 to 15 ml to avoid turbulence.

* McGraw Laboratories, Irvine, CA.
5. The number of cycles should depend on the donor’s size and reaction. Using the small bowl, six to eight cycles can be drawn. Using the large bowl, six cycles are usually satisfactory.

6. For platelets, the same donor can be used two to three times weekly since the loss of platelets corrects itself rather slowly and the half-life of the platelets is three to five days.

7. For granulocytes, the infusion should be done daily since the half-life of granulocytes is measured in hours. The same donor can be used daily since the granulocyte pool is so large that no fall in the WBC count occurs following the phlebotomy.

8. On rare occasions, a donor will complain of circumoral parathesia (citrate effect)? This can be corrected by a few sips of milk during the phlebotomy and slowing the reinfusion slightly.

9. The reinfused blood is usually cool, and the donor should be covered with a blanket if necessary. The cool sensation is uncomfortable.

Statistics

If the previous procedures are followed, yields may be expected as shown in tables I, II and figure 1.

Discussion

The use of platelets or granulocytes is made on clinical findings, rather than on absolute laboratory counts. The tolerance of patients with low platelet counts to remain free of bleeding is frequently surprising. Cases of post splenectomy refractory thrombopenia manage productive and active lives without hemorrhagic phenomena. When bleeding becomes clinically noted, or when such patients require surgery, platelet transfusions are indicated. The use of a single donor source presents many advantages, the most important being the chance of less sensitization than if multiple donor’s platelets (batch platelets) are used.

Granulocytes, likewise, are required on clinical findings rather than a reported low count. Again, asymptomatic neutropenia is frequently well tolerated. However, in the presence of infection or fever, the need for WBC becomes more imperative. The half-life of the granulocyte in the circulation is very short, and repeated infusions are needed every 12 to 24 hours. The test of the effectiveness of the infusion is the lysis of the fever or the subsidence of evidence of infection.

How to have sufficient supplies on these short-lived products available has brought investigators to the problem of storage. The success with the frozen storage of RBC’s has stimulated research in this area.

| TABLE II |
| Harvesting of White Blood Cells (WBC) & Granulocytes |

| Without hydroxyethyl starch (HES) |
| 500 ml of sterile H2O + 15 g of tri-sodium citrate |
| 32 donors |
| WBC yield = 0.24 x 10^{10} per L (total average yield = 1 x 10^{10}) |
| Polys yield = 0 (all mononuclear) |

| With hydroxyethyl starch (HES) |
| 500 ml of 6 percent HES + 15 g of tri-sodium citrate |
| 44 donors |
| WBC yield = 0.48 x 10^{10} per L (total average yield = 2 x 10^{10}) |
| Polys yield = 0.24 x 10^{10} per L (total average yield = 1 x 10^{10}) |

Average yields with and without hydroxyethyl starch.
for platelet and granulocytes. Perhaps, the necessary techniques for frozen products of this nature will soon be available.

Conclusion

This technique provides a method for the effective production of platelets and granulocytes from a single donor. The product is both adequate and functional and the technique is easily learned. The usual well equipped and staffed blood bank can absorb this procedure without major changes. The problem of longer term storage is now being studied and, hopefully, a supply of stored cellular blood components such as RBC's, WBC's and platelets will be part of our usual armamentarium.

References