REVIEW

Granulocyte Transfusions: Current Status

By Donald J. Higby and Doris Burnett

Since granulocyte transfusions first became widely used in clinical medicine, there have been advances in the treatment of acute leukemia and improvement in prevention and management of infection in neutropenic patients. Improved understanding now exists concerning prognosis of infections in such patients, and advances have been made in procurement of granulocytes. Granulocyte transfusions should be given for specific indications, and used adjunctively to other established antiinfective therapy. Once initiated, transfusions should be given in adequate doses at daily intervals (at least) with ongoing evaluation and periodic reassessment of the whole antiinfective program. Serious complications of granulocyte transfusion therapy are relatively rare, but the physician should be prepared to manage them intelligently. Research continues in discerning exactly how granulocyte transfusions work, in preservation of granulocytes, and in delineation of immunologic phenomena affecting the efficacy of such therapy. Granulocyte transfusions will continue to be important in the management of acute leukemia, and other reversible bone marrow failure states, and in marrow transplantation and autotransplantation.

CAREFULLY conducted animal experiments\textsuperscript{1-3} and clinical studies\textsuperscript{4-10} have demonstrated that transfused granulocytes are capable of bacterial activity in the recipient and do influence clinical outcome. Studies in man have shown that transfused granulocytes migrate to sites of infection\textsuperscript{11} and circulate normally,\textsuperscript{12,13} and the actual process of in vivo phagocytosis of organisms has been observed.\textsuperscript{14} Thus, investigators of granulocyte transfusion therapy no longer are preoccupied in demonstrating whether they work. The problem now is to learn to optimize the use of this new tool of clinical medicine.

Many commonly held concepts about granulocyte transfusion therapy must be reevaluated in light of recent advances in the treatment of diseases, such as acute myelocytic leukemia, in newer approaches to antibiotic therapy and supportive care for patients at high risk of bacterial infection, and in fact, in procurement methodology itself. For example, newer treatment regimens reduce the time at risk for patients undergoing remission induction for acute leukemia,\textsuperscript{15} thus per se reducing the frequency and severity of neutropenia-related infection. Currently used antiinfective regimens reduce the time at risk for patients undergoing remission induction for acute leukemia,\textsuperscript{15} thus per se reducing the frequency and severity of neutropenia-related infection. Currently used antiinfective regimens have a broader spectrum of activity than those in wide use at the time granulocyte transfusions were first being tested. Intestinal antibiotic,\textsuperscript{16,17} isolation procedures,\textsuperscript{18} the use of agents to stimulate earlier marrow recovery in chemotherapeutically ablated patients,\textsuperscript{19} and many other advances strongly suggest that if a certain fraction of acute leukemia patients with transient neutropenia absolutely required granulocyte transfusions to survive in 1970, that fraction will be less in 1980. On the other hand, there are still patients who expire from overwhelming infection during neutropenia, and with the resurgence of interest in autologous marrow transplantation to protect patients undergoing otherwise lethal therapy, it is likely that risk and thus need for granulocyte transfusions will increase.

This review is oriented towards current indications for granulocyte use, methods of procurement, donor considerations, and evaluation of efficacy.

INDICATIONS

A review of the literature strongly suggests that only in neutropenic patients with documented septicemia have granulocyte transfusions been definitely shown to improve survival.\textsuperscript{17,19} These conditions were requirements for Grav's and Herzig's studies, and such patients constituted the subgroup with the clearest difference between treated and nontreated patients in Vogler's study. With respect to infections not accompanied by septicemia or infections with nonbacterial organisms, the contribution of granulocyte transfusions to survival is not so clear. In Fortuny's study, both control and treated groups did equally well;\textsuperscript{6} in fact, their control group had a much higher survival rate than did the control groups of other studies.\textsuperscript{20} These patients for the most part did not have evidence of septicemia. Examination of these studies leads to the question whether, in those patients who would have survived anyway, granulocyte transfusions contribute materially to a reduction in morbidity, and whether this contribution justifies the added cost. As for prophylaxis, Clift found that, although his treated group had a statistically significant reduction in the
rate of infection acquisition, no significant impact on mortality was noted.\(^3\) Thus, while there may be merit in using granulocyte transfusions for indications other than bacterial septicemia in neutropenic patients, one must balance the benefits against the cost, both monetary and in terms of donor time and risk. Schiffer, for instance, has recently suggested that patients receiving prophylactic granulocyte transfusion had a high incidence of sensitization and associated reactions to other blood products.\(^2\)

In addition to the type and severity of infection, the decision to initiate granulocyte transfusions must also be based on whether the patient is expected to recover from his neutropenia, and whether, with recovery, his survival will be measurably improved. Thus, in a patient with refractory acute leukemia who acquires an infection during a period of pancytopenia, granulocyte transfusions may not be indicated. Such a decision is not arbitrary, but based on the concept that standard granulocyte support in adults is usually not associated with dramatic or complete clearing of infection, but rather with a gradual amelioration of signs and symptoms.\(^2\) If functional bone marrow is unlikely ever to recover, granulocyte transfusions will eventually become ineffective because of sensitization or infection with resistant organisms.

A word should be said about the degree of neutropenia that should prompt the attending physician to consider granulocyte transfusions, providing considerations are given to the above more important considerations. In most studies, an absolute neutrophil count of 500/cu mm was considered a requirement for entry into the study.\(^2\) This is a reasonable figure, in view of studies suggesting that the incidence and severity of infection is inversely proportional to the depth and duration of neutropenia.\(^2\) However, in the clinical setting, the overall status of the patient and the likelihood of early recovery of marrow is more important. It is a common experience to see patients begin to recover from neutropenia-related infections before the peripheral granulocyte count begins to rise. This is not surprising, since repopulation of the granulocyte compartment is noted last in the circulating pool.\(^2\) Likewise, a patient who is infected, neutropenic, but clinically stable, may be manageable with antibiotics alone. Thus, in the noncritical candidate, a 1 or 2 day trial of broad spectrum antibiotics should precede institution of granulocytes to determine whether this alone might be sufficient for infection control.\(^7\)

**Immunologic Factors**

As a minimum standard, granulocyte concentrates, which are contaminated with a significant amount of red cells, should be obtained from a donor whose red cells do not agglutinate in recipient serum. Additionally, there is reasonable evidence that posttransfusion granulocyte increments are higher the closer the HL-A match.\(^25,26\) In a nonsensitized recipient, this is probably not a limiting factor in selecting donors. However, recipients who have developed sensitization to blood products obtained from random donors should receive granulocytes from closely matched donors since (A) there is good evidence that apparent sensitization to whole blood or platelet transfusions is often directed towards contaminating white blood cells,\(^27\) (B) patients\(^25\) and animals\(^28\) who have developed sensitization to the blood products of the granulocyte donor show poor circulation kinetics and unfavorable sequestration phenomena; and (C) in sensitized patients, granulocyte transfusions have been associated with serious clinical syndromes.\(^29,30\)

The clinician usually must presumptively judge whether the recipient is sensitized to donor granulocytes, since no generally accepted test for significant antigranulocyte factors may be available. Ungerleider, in fact, was unable to correlate the presence of antileukocyte antibody (using granulocytotoxicity, lymphocytotoxicity, microleukoagglutination, and capillary leukoagglutination assays) with granulocyte recovery or the incidence of transfusion reaction.\(^31\)

In the absence of matched donors, the use of systemic short-acting steroids, antihistamines, and meperidine, together with random donor granulocyte transfusions, results in higher recovery of circulating granulocytes and a lessening of transfusion reactions. Transfusions given under such circumstances seem to be effective, and migration into skin chambers on the recipient has been demonstrated.

Recipients of granulocytes obtained by sedimentation methods have a febrile reaction rate of about 5%–10%. Other reactions are extremely rare. Filtration-procured granulocytes are accompanied by higher febrile reaction rates; while some centers have reported a rate of 40%, our own rate has decreased so that now it is about 15%.

Granulocyte transfusions procured by either method should be administered slowly, since pulmonary reactions have been reported especially in patients with established pulmonary infections.

While it is difficult to generalize regarding the causes of these reactions in all patients, one of five mechanisms can probably be implicated in the majority of cases. The first, often not considered, is simply fluid overload. Such reactions cannot clearly be documented, obviously, but we suspect this in patients who already are in congestive heart failure, who are elderly, and/or who do not have accompanying fever and chills. Measures designed to manage pulmonary
edema are rapidly effective. The second mechanism that has been documented by ourselves and others is the sequestration of granulocytes in an extensive pulmonary lesion. In a patient with a suspected pulmonary infection but with a negative chest x-ray, the administration of granulocytes may be associated with the rapid development of respiratory insufficiency, purulent sputum production, and subsequent x-ray studies compatible with a lobular or bilobular pneumonia. The third mechanism probably accounts for most severe pulmonary reactions, but is not accompanied by easily documented findings. We suspect that in many severely infected, neutropenic patients, circulating endotoxins exist (unpublished observation, also see reference 33). In the normal individual, the interaction of such substances with endothelial cells, but more importantly, granulocytes, may result in the septic shock syndrome, and/or “shock lung.” The introduction of normal granulocytes into a neutropenic patient with endotoxemia could conceivably evoke a mechanism similar to but not as severe as that described by Jacobs involving release of granulocyte enzymes, adherence of granulocytes to endothelial cells, and complement activation. Insufficient information exists to document the frequency of this sort of reaction, but in our experience, it is reversible and preventable in patients who have reacted to an initial transfusion in this manner by the administration of high doses of steroids. The fourth mechanism is that reported by Ward in which circulating leukoagglutinins cause intravascular aggregation and subsequent embolization of granulocytes into the lungs. This reaction may be the most severe, and in our experience, usually produces frank and relatively refractory pulmonary edema, with patchy bilateral infiltrates visible on chest x-ray. Finally, the fifth mechanism we have noted is the infusion of aggregated granulocytes directly. Needless to say, this last mechanism is the most easily prevented, and individuals administering transfusions of granulocytes should be carefully instructed in appropriate inspection of the transfusion and the proper use of the in-line blood filters.

In the event of pulmonary distress during a granulocyte transfusion, it is our policy to discontinue the infusion, administer 100–500 mg of hydrocortisone, intravenously, and provide symptomatic and other pharmacologic treatment. A chest x-ray should be obtained as soon as feasible, since it is sometimes helpful in terms of the differential diagnosis. The nontransfused granulocytes should be carefully inspected. A blood sample should be drawn and tested for the presence of circulating leukoagglutinins, if the capacity exists in the institution. In our own experience, no patient has ever expired due to a pulmonary reaction to granulocyte transfusions. In most cases, symptomatic relief is rapidly achieved.

If a patient has had a pulmonary reaction, studies suggested by the above analysis should be performed. In the event that no obvious preventable cause can be determined, future transfusions should be administered very slowly and preceded by premedication with intravenous steroids. Very close and careful observation of such patients is, of course, indicated.

PROCUREMENT

A physician using granulocyte transfusions should assure himself as to the quality and quantity of the granulocytes constituting a “unit.” In some centers, granulocytes are obtained as a by-product of plateletpheresis, assayed only with a white blood cell count and, perhaps, pooled with the granulocytes from other donors. There may be delays between procurement and delivery to the patient, and intercurrent storage may be suboptimal. The physician who has been disappointed with the response of his patients to granulocyte transfusions should consider that studies reporting their efficacy were performed with careful attention to the quality and quantity of the product.

There is inferential evidence that, in an adult, at least 10^10 functional granulocytes per day must be administered to be assured of a therapeutic effect. In contrast, the normal rate of production in an adult is in excess of 10^11 cells per day and can rise several-fold in the face of stress. Procurement techniques currently available result in granulocyte doses of between 0.8 and 3.5 x 10^10 cells per normal donor in 2–4 hr.

Granulocytes are obtained by methods depending on differential sedimentation or reversible adhesion. Table 1 compares various systems based on our own experience (We recognize that others might disagree with our findings and that this table should be considered a very rough guide.)

The differential sedimentation methods do not functionally alter the granulocytes and exert minimal trauma on donor blood. Granulocytes procured by these methods are indistinguishable from those obtained by simple venipuncture in virtually all parameters studied. Commercially available sedimentation systems include continuous and intermittent flow centrifugation devices.

Differential sedimentation systems result in unsatisfactory yields of granulocytes unless the process is augmented. Pretreatment of the donor with steroids and the use of hydroxyethyl starch as an additive to increase roleux formation of red cells can markedly increase the efficiency of the procedure.

Djerassi has recently described a sedimentation system that can achieve comparable amounts of gran-
Table 1. Comparison of Granulocyte Procurement Techniques: One Center's Experience

<table>
<thead>
<tr>
<th>Product</th>
<th>Storage</th>
<th>Cost/Procedure*</th>
<th>Yield/Procedure x 10^9 WBC</th>
<th>Percent* Granulocytes in Product</th>
<th>Red Cell Mass Lost/Procedure (ml Packed Red Cells)</th>
<th>Donor Reactions (Approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible adhesion</td>
<td>Variable</td>
<td>Poor</td>
<td>32</td>
<td>2.5</td>
<td>&gt;95</td>
<td>15</td>
</tr>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sedimentation</td>
<td>Intermittent flow‡</td>
<td>Good</td>
<td>Adequate§</td>
<td>30</td>
<td>1.8</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Continuous flow‡</td>
<td>Good</td>
<td>Adequate</td>
<td>55</td>
<td>1.5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Gravity‡</td>
<td>Good</td>
<td>Adequate</td>
<td>65</td>
<td>1.8</td>
<td>80</td>
</tr>
</tbody>
</table>

*Approximate means based on our standard procedures.
‡With steroids and hydroxyethyl starch.
§May be stored under proper conditions for 24 hr with <10% loss of chemotactic activity.

ulocytes (as compared to commercial systems based on centrifugation) using standard equipment available in most blood banking facilities. Our experience with this system has been favorable, but we have felt that the system becomes comparable, in terms of cost and time, to commercial systems only when the donor has a large blood volume and good venous access.

Procurement of granulocytes based on their property of reversible adhesion to fiber surfaces (filtration leukapheresis) has been shadowed by controversy since first described by Djerassi. There was evidence that with the methodology first described, granulocytes so procured did not circulate normally, had decreased chemotactic ability, and showed defects in enzymatic reactions, bactericidal reactions, etc. Such cells rapidly lose activity after procurement and should not be stored for any length of time. However, further investigation suggested that the population of granulocytes obtained during filtration leukapheresis is not homogenous. Those cells that elute in the first fraction are indistinguishable from normal, whereas those eluted in later fractions are damaged, as indicated by morphological and functional parameters. The normal fraction can be increased by a variety of methods. Steroid treatment of the donor (4 mg/sq M dexamethasone i.v. immediately before the procedure or prednisone 30–50 mg p.o. the evening before the procedure) improves the percentage of normally functioning granulocytes and reduces donor and recipient reactions. Lidocaine in the eluting fluid reduces the percent recovery of abnormal granulocytes, as does pretreatment of the donor with colchicine. In our hands, a carefully defined solution containing EDTA and dextrose resulted in more normally functioning granulocytes from filtration leukapheresis than did an ACD-plasma solution. Finally, Buchholz has recently confirmed that the centrifugal force at which the granulocytes recovered by filtration leukapheresis are concentrated is related to the degree of malfunction evidenced by recipient reactions. Thus, while filtration leukapheresis procedures can cause, at worst, morbidity in both donor and recipient, and perhaps no beneficial effect to the patient, under carefully defined conditions, given even the present level of technologic development, a nearly normal product with minimal reaction problems can be produced.

Some investigators have offered convincing evidence that the process of filtration leukapheresis results in the activation of complement in the blood returning to the donor. In fairness, it should be noted that similar changes have been noted with centrifugation methods of leukapheresis. Nevertheless, the significance of this can be questioned in view of the fact that several thousand normal individuals have undergone filtration leukapheresis without serious reactions. However, there have been reports of febrile and allergic reactions in donors, as well as rare reports of severe cramping abdominal pain, noted especially in women. Two cases of priapism have also recently been attributed to the procedure (unpublished observations). Whether such reactions are related to
complement activation is not clear; we feel that the incidence of donor reactions of the more common, mild, and self-limited type are to a large extent preventable by premedication of the donor with steroids.47

Our own filtration system requires about 2 hr to yield as many granulocytes as a centrifugation procedure does in 3–4 hr. The granulocytes obtained with filtration are not significantly contaminated by lymphocytes or platelets. The procedure results in the loss of insignificant amounts of hemoglobin compared to sedimentation procedures.

Donors subjected to leukapheresis by either sedimentation or filtration run risks not associated with ordinary pheresis procedures, such as red cell, plasma, and platelet procurement. The risks of single doses of steroids are probably minimal, but not absent; the short-term effects of hydroxyethyl starch involve symptoms of increased intravascular volume which readily dissipate. However, long-term effects are not well understood. Citrate toxicity, while probably harmless, is extremely disconcerting to donors, especially when anxiety-induced hyperventilation complicates the hypocalcemia. Anticoagulation, whether with heparin or citrate solutions, carries its own risk; too little anticoagulation could in theory cause intravascular coagulation as a result of the triggering of the coagulation mechanism by the direct pathway in the reinfused blood. In any system using a roller pump to move blood, intravascular hemolysis by trauma due to improperly fitted tubing can occur. Finally, complement activation probably carries some risk. The purpose of this discussion is not to dwell in depth on donor risks and treat the resulting product with the respect it deserves.

Transfusiologists prepared to carefully monitor the procedure and the product would not be amiss in using filtration leukapheresis. Without such care, however, it is probably better to select a centrifugation procedure.

ASSESSMENT OF RESPONSE

While resolution of a pulmonary infiltrate, abscess, or other localized infection permits an objective assessment of the success of combined antiinfective therapy in the neutropenic patient, upwards of 40% of febrile neutropenic patients have no clinically evident source of infection. In such situations there may be improvement in clinical parameters, such as appetite, state of alertness, average height of fever, etc., or stabilization following a downhill course may be observed. In most studies, four to nine daily transfusions were given per patient episode.20 After initiating granulocyte transfusion therapy, it is reasonable to continue support for 7 days before modifying the therapeutic program.

It is important to periodically reassess the entire antiinfection equation. The addition of antifungal agents empirically may be indicated. If improvement is not evident after several daily "standard" granulocyte transfusions, doubling the dose or increasing the frequency has favorably influenced the course in some apparently refractory patients, in our experience.

PROSPECTS FOR THE FUTURE

(A) The ability to determine whether a febrile neutropenic patient is likely to recover without granulocytes would permit much better utilization of this expensive and scarce resource. While it is probably no great loss to administer granulocyte transfusions to those who would recover anyway, to use "megatransfusions" in those who are extremely ill might result in a decrease in mortality in this smaller group.

Studies are being conducted in neutropenic infected patients, in which the relative weights (by mathematical modeling techniques) of clinical and laboratory parameters obtained early in the course of the infection, are used to determine whether it is possible to predict more accurately which patients might do well with antibiotic therapy alone as opposed to those in which granulocyte transfusions and other vigorous supportive measures might be more rationally introduced at an earlier stage. Likewise, studies are being performed to determine whether some separation of these two categories of patients can be made on the basis of parameters such as the behavior of normal granulocytes in recipient serum, the presence of circulating endotoxin, and the in vivo behavior of an aliquot of transfused granulocytes. A better delineation of indications will permit a more rational approach to antinfective therapy, including granulocyte use, in neutropenic and infected individuals.

(B) Better methods of procurement are clearly needed. The limiting factor in either procurement method is the time a normal donor can afford to spend undergoing the procedure. Yet, it is reasonable to assume that a fivefold increase in the number of granulocytes per transfusion would result in a better clinical result and probably make the prophylactic use of granulocytes more practical.

(C) A more reliable system for measuring anti-granulocyte antibodies would permit better pairing of donors and recipients. While probable "mismatches" in this system do not usually result in any great direct harm, properly matched granulocytes would improve the usefulness of such transfusions.
GRANULOCYTE TRANSFUSIONS

(D) We believe that granulocyte transfusions in the doses given currently may result in positive clinical results through mechanisms related to synergism with antibiotic therapy and residual host defenses, rather than solely through their native antipathogenic properties. A better understanding of exactly how these physiologically minimal doses of granulocytes actually exert a beneficial effect would permit the exploration of methods to augment these mechanisms.

(E) Successful cryopreservation of granulocytes would of course be ideal. Patients with chronic myeloid leukemia could be leukapheresed extensively prior to treatment so that their functionally normal cells could be used for many courses of granulocyte transfusion therapy. Currently, some success has been achieved on an experimental basis towards this desirable end.37

CONCLUSIONS

With more intensive therapy regimens for acute myelocytic leukemia and with resurgent interest in autologous transplantation as part of approaches to solid tumor chemotherapy, there will continue to be a demand for safe and effective granulocyte transfusions. Research must be directed towards improving techniques for obtaining, preserving, and using them more judiciously.

ACKNOWLEDGMENT

I am grateful to Corrine Cesari for preparing this manuscript with skill and patience.

REFERENCES

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DJ Higby and D Burnett