Granulocyte transfusions: a review

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Granulocyte transfusions have been used for over 25 years to treat neutropenic patients with severe bacterial or fungal infections that are unresponsive to antimicrobial therapy. Methods to mobilize and collect granulocytes have changed, but the use of granulocyte transfusions has not increased. The availability of hematopoietic growth factors that markedly increase donor granulocyte counts, collection yields, and possibly clinical efficacy has resulted in renewed interest in these transfusions. Clinical trials of growth factor-mobilized granulocytes are being planned or are under way. As the results of these studies become known, use of these components is likely to increase.

Indications for Granulocyte Transfusions
Several studies have found that granulocyte transfusions can benefit neutropenic patients who have bacterial infections not responsive to antibiotic therapy. They are more likely to be beneficial when the duration of neutropenia is prolonged, the risk of death is high, larger doses are transfused, and attempts are made to give compatible cells. Although some studies have not shown that granulocyte transfusions confer a survival benefit, this result is due to low cell doses and to a failure to provide compatible cells. Trials of granulocyte transfusions to prevent infections in neutropenic patients have not been successful, but a meta-analysis of the efficacy of prophylactic granulocyte transfusions indicated that further trials using higher cell doses and crossmatch-compatible cells might be effective.

Mobilizing Granulocytes
In order to increase the number of cells that can be collected by apheresis, granulocyte donors are generally given an agent to increase their neutrophil count. Steroids have been the standard agents, but leukocyte growth factors have been found to induce a greater increase in granulocyte counts and are increasingly being used in addition to or in place of steroids.

Steroids
Dexamethasone and hydrocortisone have been used to increase the cell counts of granulocyte donors. Steroids increase the granulocyte count by releasing cells from storage in margined pools and from the marrow. Granulocyte counts peak approximately 4 to 6 hours after an oral dose of steroid is given and remain at peak levels for at least 24 hours. An 8-mg dose of dexamethasone doubles both the granulocyte count and the number of granulocytes collected by apheresis (Table 1).

Table 1. Effects of mobilizing agents on granulocyte counts and granulocyte concentrate yields

<table>
<thead>
<tr>
<th>Mobilizing Agent and Dose</th>
<th>Average Granulocyte Count (× 10^9 cells/L)</th>
<th>Average Apheresis Yield (× 10^10 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6 to 9</td>
<td>2</td>
</tr>
<tr>
<td>300 µg or 5 µg/kg</td>
<td>16 to 26</td>
<td>3 to 5</td>
</tr>
<tr>
<td>300 µg or 5 µg/kg</td>
<td>29 to 34</td>
<td>6 to 8</td>
</tr>
<tr>
<td>600 µg</td>
<td>None</td>
<td>27</td>
</tr>
<tr>
<td>600 µg</td>
<td>8 mg</td>
<td>45</td>
</tr>
</tbody>
</table>

*The numbers given refer to mean blood counts and apheresis yields; the range shown is the range in means reported by different investigators. N.D. = no data

Granulocyte colony-stimulating factor
Granulocyte-colony-stimulating factor (G-CSF) also releases granulocytes from marginated pools and marrow. Within 30 minutes after the subcutaneous injection of the G-CSF, the granulocyte count falls. This granulocytopenia is transient, and after 2 to 4 hours, the granulocyte count returns to normal and then increases. The granulocyte count peaks about 12 hours after G-CSF is given, remains markedly elevated for 24 hours, and after 72 hours returns to normal. Initially, G-CSF increases the granulocyte count by releasing marginated granulocytes from the spleen, but after 24 hours much of the increase is due to the release of cells from the marrow.

Kinetic studies found that a single G-CSF dose of 300 µg increased the absolute neutrophil count (ANC) sevenfold over baseline to 25 × 10^9/L after 12 hours. Little further increase in granulocyte counts occurred if a higher dose of 600 µg was given. Both G-CSF and dexamethasone have been used together, and this combination mobilizes significantly more granulocytes. When 300 µg of G-CSF and 8 mg of dexamethasone are given in combination, the ANC increases ninefold (Table 1).

Other investigators have found that the administration of 5 µg/kg of G-CSF to granulocyte donors the day
before donation increased their ANC four- to five-fold.6,9–11

**Granulocyte-macrophage colony-stimulating factor**

Granulocyte-macrophage colony-stimulating factor (GM-CSF) appears to be less effective than G-CSF in mobilizing granulocytes and is used less frequently in granulocyte donors. A single dose of 5 μg/kg of GM-CSF increases granulocyte counts in healthy donors about twofold.12 The effect of giving GM-CSF and dexamethasone in combination has not been reported.

**Effects of Granulocyte Mobilizing Agents on Donors**

The agents used to mobilize granulocytes may create logistical as well as physical problems for donors. The need to give a parenteral mobilizing agent is inconvenient. The donor must come to the apheresis center on two separate occasions, once to be given the drug and again to undergo the procedure. Steroids are taken orally and are self-administered, but G-CSF and GM-CSF must be given as a subcutaneous injection at the apheresis center or clinic.

**Symptoms**

An 8-mg dose of dexamethasone caused 44 percent of donors to experience insomnia or flushing.6 The effects of a single G-CSF dose are slightly more severe. After receiving a 5 μg/kg dose of G-CSF, 68 percent of donors experienced bone pain, headaches, insomnia, or fatigue.6 This increased to 72 percent in donors given both G-CSF and dexamethasone. Data are not available concerning the effects of a single dose of GM-CSF on donors. However, when donors were given GM-CSF for 5 days to mobilize hematopoietic progenitor cells, their symptoms are similar to those experienced by donors given G-CSF.12

**Blood chemistries and counts**

Several changes in blood counts and blood chemistries occur in donors peripheral blood stem cells (PBSCs) during and after their collection. These changes include mild transient thrombocytopenia and neutropenia; increases in blood levels of alkaline phosphatase, lactate dehydrogenase (LDH), and uric acid; and decreases in bilirubin, sodium, and potassium.13–16 Donors of PBSC concentrates are given G-CSF for 4 to 6 days, but granulocyte donors get a single dose. The exact effects of a single dose on blood chemistries are not certain, but it is likely to increase alkaline phosphatase and LDH. The other blood chemistry changes in PBSC donors are of lesser magnitude and are less likely to occur in granulocyte donors. It is not certain if granulocyte donors will experience the mild neutropenia or thrombocytopenia that has been noted in PBSC donors.

**Collection of Granulocytes**

In the past, granulocytes were collected by filtration or centrifugation devices from healthy volunteer donors or patients with chronic myelogenous leukemia. Currently, granulocytes are only collected from healthy donors by use of centrifugal blood cell separators. For a typical granulocyte collection, 7 L of blood are processed over 2 hours.

A red cell sedimenting agent is usually added to the donor’s whole blood as it enters the blood cell separator to enhance the separation of granulocytes from red blood cells (RBCs) and to increase the granulocyte yield. Hydroxyethyl starch is the most commonly used sedimenting agent, and it is available in two formulations: as hetastarch or as pentastarch. Pentastarch has a lower molecular weight and is cleared faster from the donor’s blood than is hetastarch, but the process of granulocyte collection is more efficient when hetastarch is used. There was a 64 percent improvement in granulocyte yields in dexamethasone-treated donors when hetastarch was used compared with pentastarch (2.3 ± 0.67 × 10^10 versus 1.4 ± 0.76 × 10^10 granulocytes per component in 72 sets of paired donations).17

Depending on the mobilizing agents used, it is now possible to routinely collect 2 to 10 × 10^10 leukocytes per leukopheresis, of which 85 to 95 percent are granulocytes. When dexamethasone is used to mobilize granulocytes, 2 × 10^10 can generally be collected. When G-CSF alone is used to mobilize granulocytes, 3 to 5 × 10^10 cells can be collected. When both G-CSF and dexamethasone are given, 6 to 8 × 10^10 granulocytes are harvested (Table 1). Data are not available as to how many granulocytes can be collected from donors given GM-CSF.

Granulocyte concentrates contain a large number of platelets and RBCs. Even when a RBC sedimenting agent is used, the granulocyte concentrate contains about 30 mL of RBCs. Thus, ABO compatibility must be considered in recruiting granulocyte donors for specific patients. Granulocyte concentrates contain about 4 × 10^11 platelets, a quantity similar to an apheresis platelet concentrate.

**Storage of Granulocytes**

Granulocyte concentrates may be stored for as long as 24 hours at 20°C to 24°C.18 However, after 24 hours of
room-temperature storage, in vitro chemotaxis, and in vivo intravascular recovery, and migration into experimental skin blisters are markedly decreased. For these reasons, granulocytes should be transfused as soon as possible after collection.\textsuperscript{19,20} Storing granulocytes at 1°C to 6°C better preserves some cell functions\textsuperscript{19} but not intravascular recovery or migration into skin blisters.\textsuperscript{20}

**Function of Transfused Granulocytes**

**Steroid mobilized granulocytes**

The chronic administration of steroids inhibits a number of granulocyte functions, including adherence to surfaces, chemotaxis, phagocytosis, degranulation, generation of superoxide, and antibody-dependent cytotoxicity.\textsuperscript{21-24} However, these effects do not occur at the doses given to granulocyte donors. Granulocytes collected from donors treated with a single 8-mg dose of dexamethasone have normal chemotaxis, phagocytosis, bacterial killing, and accumulation into experimental skin blisters.\textsuperscript{25,26}

**G-CSF-mobilized granulocytes**

G-CSF has several effects on the structure, function, and life span of granulocytes. Many changes in the expression of granulocyte antigens follow the administration of G-CSF.\textsuperscript{7} G-CSF stimulates granulocytes, and within 30 minutes the expression of several granulocyte antigens increases (Table 2). The expression of most anti-gens returns to normal after 4 hours, but the expression of FcγRIIIb (CD16) falls below baseline levels after 4 hours and remains low after 12 and 24 hours. In addition, 12 to 24 hours after G-CSF is given, the expression of CD14 and FcγRI (CD64) rises, but the expression of L-selectin (CD62L) falls.

Despite the changes in granulocyte antigen expression in vitro, the granulocytes collected from individuals given G-CSF show normal chemotaxis and phagocytosis but elevated superoxide production, chemiluminescence, and antibody-dependent cytotoxicity.\textsuperscript{10,26} The fall in expression of the most abundant Fc receptor on neutrophils, FcγRIIIb, which is induced by G-CSF, probably does not affect the function of these cells significantly. Individuals with congenital deficiency of FcγRIIIb are not at increased risk of bacterial or fungal infections.\textsuperscript{27} In addition, the expression of another Fc receptor, FcγRI, is increased by G-CSF.

L-selectin is an important mediator of interactions between neutrophils and endothelial cells, but the G-CSF-induced fall in the expression of L-selectin does not appear to have a significant detrimental effect on granulocyte function. In vivo studies have found that the migration of G-CSF-mobilized granulocytes to sites of inflammation was unimpaired in a study of allogenic marrow transplant recipients.\textsuperscript{28}

Paradoxically, the reduction of L-selectin expression could make the transfusion of these granulocytes more effective rather than less effective. Preliminary studies have found that the in vivo half-life of G-CSF-mobilized granulocytes is 8 to 24 hours, compared with 5 to 6 hours for granulocytes mobilized with only dexamethasone.\textsuperscript{9,11,20,29,30} The prolonged circulation of G-CSF-mobilized granulocytes may partly be due to the decrease in L-selectin expression. In addition, immediately after being transfused, most transfused granulocytes become transiently trapped in the lungs.\textsuperscript{31} If this pulmonary sequestration of granulocytes is mediated by L-selectin, then granulocytes from G-CSF-treated donors might have a greater and a more immediate posttransfusion intravascular recovery and be less likely to be trapped in the lungs and cause pulmonary reactions. Lastly, G-CSF has been shown to suppress neutrophil apoptosis or programmed cell death, which also might contribute to prolonged survival of G-CSF-mobilized cells in vivo.\textsuperscript{32}

**Survival and Trafficking of Transfused Granulocytes**

When dexamethasone-mobilized granulocytes are transfused, little or no change in the neutrophil count occurs in the transfusion recipient.\textsuperscript{9} In contrast, when granulocytes mobilized with G-CSF or G-CSF plus dexamethasone are given, the recipient's neutrophil count increases by 0.4 to 1 × 10\textsuperscript{9} cells/L 1 hour after the transfusions and remains elevated for the next 24 hours.\textsuperscript{9,11,29}

In addition, G-CSF-mobilized granulocytes remain in the circulation longer than dexamethasone-mobilized cells. The exact mechanism for their prolonged survival is not certain, but G-CSF retards the programmed cell
death, or apoptosis, of granulocytes. This is likely to be responsible for at least part of the prolonged survival of G-CSF-mobilized cells. In addition, G-CSF may induce the release of immature granulocytes from the marrow, and this also could contribute to their longer survival.

**Alloimmunization**

Although the treatment of donors with G-CSF has resulted in a marked increase in the number of granulocytes that can be collected, alloimmunization may limit the use of granulocyte transfusions in many patients. Multiparous women and transfusion recipients are often alloimmunized to human leukocyte antigens (HLA). The rate of alloimmunization in patients given granulocyte transfusions is as high as 60 to 80 percent. The proportion of patients who become alloimmunized increases with the number of granulocyte transfusions.

The transfusion of granulocytes to alloimmunized patients is complicated by granulocyte transfusion recipients often producing antibodies to granulocyte-specific antigens in addition to antibodies to HLA. In one study, 10 of 14 patients who produced HLA antibodies also produced antibodies to granulocyte antigens. The antibodies reacted with granulocyte-specific antigens NA1, NA2, and NB1, and other antigens on Fc-γRIIIb and CD18.

**Granulocyte Transfusions in Alloimmunized Patients**

The production of antibodies to HLA and granulocyte-specific antigens can be clinically important in granulocyte transfusion recipients. These antibodies can induce transfusion reactions and render the transfused granulocytes ineffective.

**Reactions to granulocyte transfusions**

The transfusion of granulocytes to patients who have been alloimmunized to HLA or granulocyte-specific antigens results in febrile and occasionally pulmonary transfusion reactions. In one study, up to 90 percent of patients who were alloimmunized had adverse reactions related to granulocyte transfusions, whereas 11 percent of transfusion recipients that were not alloimmunized had no reactions. In a second study, 11 of 14 alloimmunized patients experienced febrile reactions, but none of 4 patients who were not alloimmunized experienced reactions. If the transfusion recipients are treated with antipyretics prior to transfusion, in most cases the febrile reactions will be well tolerated.

Pulmonary reactions associated with the granulocyte transfusions can be more severe; these include chills, dyspnea, chest tightness, acral cyanosis, and hypoxia. The hypoxia can be severe enough to require the use of supplemental oxygen, but assisted ventilation is rarely required. The pulmonary reactions can take up to 12 hours to resolve. These reactions differ from transfusion-related acute lung injury (TRALI; Table 3). TRALI is due to a reaction between the leukocyte antibodies in the transfused blood component and the transfusion recipient’s leukocytes. Pulmonary reactions in granulocyte transfusions are the result of reactions between leukocyte antibodies in the transfusion recipient and the transfused granulocytes. TRALI is clinically more severe, and the patients experiencing these reactions often require the support of a ventilator. The hypoxia can last 24 hours or longer. Although pulmonary reactions associated with granulocyte transfusions are usually transient, the transfusions should be discontinued if a pulmonary reaction occurs.

**Antibodies and granulocyte trafficking**

When leukocyte antibodies react with transfused granulocytes, the recovery of the transfused cells is decreased, their intravascular survival is reduced, and they fail to migrate into skin blisters and sites of infections. One study found that indium 111-labeled granulocytes trafficked into sites of infection in 20 of 20 non-alloimmunized patients studied but failed to do so in 11 of 14 alloimmunized patients. Another study found that the presence of leukocyte antibodies in the transfusion recipient was associated with decreased recovery and half-life of transfused granulocytes.

**Granulocyte matching and crossmatching**

Because recipients of granulocyte transfusions can produce antibodies to both HLA and granulocyte-specific antigens, the transfusion of alloimmunized patients with granulocytes from HLA-identical donors is sometimes not effective. Because the granulocyte antibodies
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may react with antigens other than to the well-characterized NA1, NA2, and NB1 antigens, the transfusion of granulocytes matched for HLA and granulocyte NA1, NA2, and NB1 antigens may not always be effective. In principle, crossmatching can be used to identify compatible granulocyte concentrates for alloimmunized patients. Ducher and colleagues found that compatibility in leukoagglutination and lymphocytotoxic crossmatches was predictive for migration of granulocytes into sites of infection. McCullough and colleagues found that the detection of leukocyte antibodies by granulocyte agglutination or immunofluorescence assays was predictive for survival and tissue infiltration by transfused cells. Granulocyte agglutination was the most useful assay. Unfortunately, the logistics of collecting granulocytes makes the use of crossmatch-compatible granulocytes difficult. One strategy to find crossmatch-compatible platelets is to test patient serum against cryopreserved platelets from potential apheresis donors. If compatible platelets are found, the donor is recruited to donate platelets by apheresis. Unfortunately, adequate methods to cryopreserve granulocytes are not available. Other strategies to obtain crossmatch-compatible platelets involve crossmatching the patient’s serum against platelets from apheresis concentrates in the blood center’s or blood bank’s general inventory. However, because few patients at any one time require granulocyte transfusions, blood banks seldom have multiple granulocyte concentrates available that can be crossmatched.

An alternative strategy is to test sera from all potential granulocyte transfusion recipients for antibodies to HLA antigens before their first transfusion. If the patient is making multiple or broadly reactive HLA antibodies, then no transfusions should be given because the transfusions are not likely to be effective and serious pulmonary reactions could occur. If the patient is producing antibodies to only one or two HLA antigens, then a trial of HLA-compatible transfusions is warranted. If the transfusion of HLA-compatible granulocytes causes a reaction or does not increase granulocyte counts, then the patient's sera should be tested for granulocyte-specific antibodies or the transfusions should be discontinued.

RBC Antigens and Antibodies

Because granulocyte concentrates contain approximately 30 mL of RBCs, the ABO and Rh type of the granulocyte donor and recipient must be compatible. In addition, if the recipient has produced antibodies to any other clinically significant RBC antigens, then the granulocyte donor’s and the recipient’s RBCs must be matched for these antigens. Methods have been described for using hetastarch sedimentation to remove RBCs from granulocyte concentrates collected by apheresis. Approximately 90 percent of RBCs can be removed with a loss of only about 20 percent of the granulocytes.

Amphotericin

The concurrent administration of amphotericin B and granulocyte concentrates has been associated with severe pulmonary reactions. It is thus advisable to allow several hours to pass between the transfusion of granulocytes and the infusion of amphotericin B.

Cytomegalovirus

Granulocyte concentrates, like any other cellular blood component, can transmit cytomegalovirus (CMV). The transmission of CMV can be prevented only by selecting seronegative donors to support seronegative patients.

Irradiation of Granulocytes

Granulocyte concentrates contain a large number of contaminating lymphocytes and must be irradiated before they are transfused to immunocompromised patients at risk for graft-versus-host reactions. Doses of radiation below 2500 cGy do not appear to impair granulocyte function.

Logistics of Collection and Transfusion

The use of granulocyte transfusions is limited by difficulties with collection and storage. The infrequent use of these components, their short shelf life, and the need for donors to make two visits to the apheresis center make the daily transfusion of these components difficult. Granulocyte concentrates should be transfused as soon as possible and not more than 24 hours after collection. The inability to store the granulocytes for longer periods prevents the collection of extra granulocyte concentrates to give to a patient should a scheduled donor be unable to donate. Mobilization schedules involving G-CSF and/or dexamethasone given the day before collection prevents the immediate collection of a substitute mobilized concentrate when a donor is unavailable the day of the collection. The inability to maintain an inventory of granulocyte concentrates also prevents the selection of crossmatch-compatible or phenotype-compatible concentrates for alloimmunized recipients from an inventory of granulocyte concentrates.
Conclusions

Dexamethasone-mobilized granulocyte concentrates are likely to be helpful in treating severe bacterial infections in neutropenic patients. The small quantity of granulocytes in these components, however, imposes limits on their effectiveness. The administration of G-CSF to donors increases granulocyte yields by three- to fourfold, and the transfusion of G-CSF-mobilized granulocytes measurably increases the granulocyte count in the recipients. Granulocyte donors treated with G-CSF often experience bone pain, headaches, fatigue, and insomnia, but these symptoms are in general mild and short-lived. Case reports suggest that granulocytes collected from donors given dexamethasone, but the effectiveness of G-CSF-mobilized granulocyte transfusions has not yet been rigorously evaluated. Randomized, prospective trials of the ability of this new blood component to improve survival in neutropenic patients with life-threatening fungal and bacterial infections are clearly warranted. However, because of the increased yields with G-CSF and G-CSF plus dexamethasone, the use of granulocyte concentrates will likely increase. New clinical trials of this blood component are warranted, especially to treat neutropenic patients with fungal infections and to prevent infections in neutropenic patients. In addition, studies are needed to better document the effects on donors of G-CSF alone and in combination with dexamethasone.

References

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