Review Article

Granulocyte transfusion therapy

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Abstract: Granulocyte transfusion therapy is often used in patients with severe neutropenia after chemotherapy who develop bacterial and fungal infections not responsive to antimicrobial therapy. Granulocyte products may be obtained by a leukapheresis procedure or by pooling buffy coat layers from whole blood. Donors must be stimulated with dexamethasone with or without granulocyte-colony stimulating factor to enhance granulocyte collection yields. The dose of granulocytes transfused is key to observing any positive effects. Higher doses are more likely to lead to detectable clinical efficacy. However, several challenges exist for obtaining sufficient granulocytes in each product. Among them are the ability to stimulate donors adequately and the efficiency of the collection instruments used. To this day, there has been no definitive evidence to show the clinical efficacy of granulocyte transfusion therapy. Published studies have shown a wide range of results from inconclusive to no benefit or benefit over not using granulocyte products. A well-designed and adequately powered randomized-controlled clinical trial would provide the needed evidence to determine whether granulocyte transfusion therapy is truly beneficial. The hurdles to conducting such a clinical trial are demonstrated by the Resolving Infection in Neutropenia and Granulocyte (RING) trial and may never be overcome. More creative ways of obtaining sufficient data need to be employed.

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Introduction

Granulocytes are a type of white blood cells that are characterized by the presence of specific granules in their cytoplasm. The four types of granulocytes are basophils, eosinophils, neutrophils, and mast cells. Of these four types, the most abundant is neutrophils which comprise approximately 60% of the nucleated cells in the bone marrow and bloodstream (1). They have a circulating half-life of 6–8 hours in the blood (1). This review article will focus on neutrophil granulocytes.

Neutrophils play a central role in rapidly activating the innate immune system to clear invading bacteria and fungi. The key neutrophil functions that allow them to neutralize foreign invaders include phagocytosis, chemotaxis, enzyme/peptide secretion, cytokine/chemokine production, and reactive oxygen species production. The primary (azurophilic) granules found in immature cells contain proteins that kill bacteria and stimulate phagocytosis of IgG antibody-coated bacteria (2). The secondary (specific) granules found in mature cells contain compounds that are involved in the formation of toxic oxygen species, lysozyme, and lactoferrin (3). The significance of neutrophils in immune system homeostasis is exemplified by the significant morbidity associated with iatrogenic neutropenia caused by chemotherapy or cytotoxic drugs (1).

Granulocyte transfusions are indicated for patients with severe neutropenia characterized by an absolute neutrophil count (ANC) less than 500/μL, fever for 24–48 hours with persistent morbidity, documented bacterial or fungal infection unresponsive to antimicrobial therapy and a reasonable hope of marrow recovery (4).

Apheresis granulocytes are not licensed products by the United States Food and Drug Administration so no

published regulations exist. American Association of Blood Banks has published standards for granulocytes that require the red blood cell (RBC) content in apheresis granulocytes to be ABO and crossmatch compatible with the recipient's plasma unless there are fewer than 2 mL of RBCs and to have a minimum of 1×10^{10} granulocytes in at least 75% of the units tested unless they are prepared for neonates (4).

Leukapheresis granulocytes

Granulocytes are collected using a leukapheresis procedure after the donor is stimulated with an adrenal corticosteroid such as dexamethasone and/or granulocyte-colony stimulating factor (G-CSF). In the absence of stimulation, healthy donors possess low levels of circulating granulocytes. Granulocyte products collected by apheresis from unstimulated donors have yields between 0.1 and 1×10^{10} granulocytes which are not typically sufficient to cause significant changes in the patient (4,5). Studies have shown that the dose of granulocytes administered is a very important factor for patients to receive any benefit. This is one of the biggest challenges for granulocyte transfusions because the normal human circulating neutrophil pool is 30×10^{7}/kg and the daily therapeutic dose suggested by canine experiments is $>15\times10^{7}$/kg (6).

When donors are stimulated for 8–16 hours prior to donation with dexamethasone and/or G-CSF, leukapheresis yields have been as high as 4×10^{10}–8×10^{10} or more, depending on the G-CSF dose and administration schedule (4,7-10). Products collected from donors stimulated with steroids alone contain less granulocytes (1×10^{10}–2.5×10^{10}) than products collected from donors stimulated with both steroids and G-CSF (4,7-9).

Corticosteroids enhance granulocyte collection yields by increasing marrow release of granulocytes, decreasing granulocyte efflux from the blood and reducing the circulating lymphocyte count (6,11). The typical dose used is 8mg taken by mouth 12–24 hours prior to leukapheresis. This is associated with few and tolerable side effects (6,12).

G-CSF is a glycoprotein secreted by various cells such as bone marrow stromal cells, macrophages, fibroblasts and endothelial cells that induces the bone marrow to produce neutrophilic granulocytes (6,13). G-CSF stimulates the proliferation and differentiation of neutrophil colony-forming cells and alters several functions of mature neutrophils when tested *in vitro* (13,14). The clinical use of G-CSF has led to few reported side effects which include bone pain, headache, fatigue, nausea and occasionally fever. This excellent safety profile has encouraged its use in healthy donors; however, concerns remain (6). Leukostasis, potential thromboembolic complications, splenomegaly and splenic rupture are among the concerns raised. A single dose of G-CSF required for mobilization and collection of granulocytes should theoretically pose no significant risk for these adverse effects but it may be prudent to limit the age of donors and exclude those with known vascular, inflammatory or autoimmune disease (6,15).

The typical volume of the granulocyte product collected is between 250 and 300 mL. Aside from granulocytes, each unit of granulocyte product contains 20–50 mL of red blood cells (approximately 10% hematocrit), about 3×10^{11} platelets, and approximately 250 mL of plasma (4). Due to the high hematocrit of these products, they must be ABO/Rh compatible, fully crossmatch compatible with the recipient, and irradiated. Granulocyte products should be transfused as soon as possible (and no more than 24 hours) after collection. Due to the urgency of the request, the results of infectious disease testing of the granulocyte donors are not usually available at the time of product issue (4). To minimize the chances of using donors with positive infectious disease markers, donor centers routinely select repeat donors (e.g., Platelet donors) with a history of negative test results within the last 10–30 days. This acceptable time frame may be extended under special circumstances; specifically, if the anticipated course of therapy will be prolonged (e.g., greater than 1 or 2 weeks), the patient has a rare blood type (e.g., B negative), and/or there are special needs [e.g., cytomegalovirus (CMV) seronegative]. Extending the acceptable time frame to expand the donor pool would require consent from the patient's physician or hospital transfusion medicine physician. Prior to the apheresis donation, the donor's medical history is obtained after which the procedure is explained and verbal consent is obtained from the donor. A prescription for the mobilization agent such as corticosteroids, G-CSF or both is sent to the donor's pharmacy along with instructions on when to take the medication prior to the leukapheresis procedure (4).

The leukapheresis procedure may take 90 to 120 minutes depending on how well the donor tolerates the procedure. Hydroxyethyl starch or another erythrocyte sedimenting agent may be mixed with trisodium citrate to allow differential centrifugation of the anticoagulated blood. Approximately 7–10 L of whole blood is processed during

each procedure (4).

Pooled granulocytes

Granulocytes may be obtained by pooling the buffy coat of whole blood donations such as is done in England. Bashir *et al.* (16) pooled 10 ABO-matched buffy coats with 400 mL of platelet additive solution followed by re-centrifugation which resulted in the development of a purer pooled granulocyte component from whole blood donations. The pooled granulocyte component they created had a similar number of neutrophils (~0.9×10^{10}) with reduced volume and hemoglobin content when compared with 10 individual buffy coats. Massey *et al.* (17) evaluated the safety of transfusing pooled, whole-blood-derived granulocytes in additive solution and plasma (GASP) in 30 recipients. GASP products contained an average of 1×10^{10} granulocytes in 207 mL or granulocyte product. They found that GASP had a similar safety profile to other sources of granulocytes for patients with refractory infection or in need of secondary prophylactic transfusion (17).

One potential disadvantage of pooled granulocyte products compared to apheresis collected granulocytes is an increased risk of human leukocyte antigen (HLA) class I alloimmunization because of the multiple donors needed to create a pooled product rather than the single donor used for an apheresis collection. In the study performed by Massey *et al.* (17), alloimmunization occurred in 10% of participants receiving GASP but more studies are needed to further characterize the risk of HLA alloimmunization posed by using pooled granulocyte products.

Transfusion

Granulocytes must be transfused as soon as possible but no more than 24 hours after collection to preserve their function. They should be stored at 20–24 ℃ without agitation prior to transfusion. Granulocyte products are issued under emergency release because infectious disease testing is incomplete at the time of issue. Granulocytes must be irradiated to prevent transfusion-associated graft versus host disease and infused through a standard blood filter (170 μm). CMV-seronegative donors must be recruited to donate granulocytes for CMV-seronegative patients since leukocyte reduction cannot be used to create CMV-safe products. HLA-matched donors may also be required for patients with a high level of alloimmunization.

Granulocyte transfusions should be discontinued when the ANC is greater than 500/μL for 2 days, clinical response is observed, no donors are available, severe transfusion reactions have occurred or when there is hopeless deterioration of the patient's condition (4).

Efficacy

The clinical efficacy of granulocyte transfusions has not been definitively shown through any high-quality clinical trials. Published studies to date have reported the gamut of responses from an improvement in outcomes for patients receiving granulocyte transfusion therapy to those in which patients did not show improvement after receiving granulocyte transfusion therapy while other studies showed inconclusive results.

Controlled trials in the 1970s and 1980s showed mixed results but generally indicated that the therapy was modestly efficacious (18). However, granulocyte transfusion therapy fell out of favor after the initial period of enthusiasm as a result of improvements in antimicrobial therapy, occurrence of adverse events, transmission of CMV, and absence of meaningful clinical improvements (19). A Cochrane review analyzing 10 trials between 1975 and 2015 found no difference in all-cause mortality over 30 days and clinical reversal of concurrent infection between study subjects receiving therapeutic granulocyte transfusions and those that did not (20). However, there are some small retrospective studies that were able to show some efficacy in patients with hematological diseases or malignancies who were neutropenic and suffered from infections unresponsive to standard antimicrobial treatment (21-23). Several uncontrolled trials performed in the 21st century that used high-dose granulocyte transfusion therapy were not able to show any conclusive clinical efficacy (24-28).

In 2015, the results of the multicenter randomized controlled trial, resolving infection in neutropenia with granulocytes (RING) trial (19), was published. This study attempted to provide conclusive evidence of the clinical efficacy of high-dose granulocyte transfusion therapy. Subjects were randomized to receive either standard antimicrobial therapy or a combination of standard antimicrobial therapy plus daily granulocyte transfusions from donors stimulated with G-CSF and dexamethasone. A total of 114 subjects were randomized to 2 groups: 58 subjects in the control group and 56 subjects in the granulocyte group. Subjects who received granulocyte therapy received 5 transfusions with a mean transfusion dose of 54.9×10^{9} granulocytes. There was no statistically

significant difference in overall success rates between the 2 groups (42% granulocyte group, 43% control group, P>0.99) and for subjects who received their assigned treatments (49% granulocyte group, 41% control group, P=0.64) (19).

There were several limitations that should raise caution when interpreting the results of the study. The first is that accrual rates were low due to a number of factors. Less than half of subjects that were necessary to provide 80% power if the true success rates in the control and transfused groups were 50% and 70%, respectively, were enrolled. With an enrollment of 114 out of 236, there was only ~47% power to detect that difference (19). The second is that the dose of the granulocytes transfused did not meet the minimum requirement of the study (4×10^{10}, 0.6×10^{9} per kg for a 70 kg subject) for more than one quarter of the subjects. It is possible that the success rates would have been statistically significantly different between the 2 groups if everyone in the granulocyte group had received high-dose granulocyte transfusions as planned. A secondary analysis comparing the success rate of subjects who received a mean dose of $\geq0.6\times10^{9}$ granulocytes/kg/transfusion to subjects who received $<0.6\times10^{9}$ granulocytes/kg/transfusion showed a highly significant difference (59% *vs.* 15%); however, this should be interpreted with caution because the success in the control group was intermediate between that of the low-dose group and high-dose group (19).

One of the reasons it has been difficult to enroll sufficient numbers of subjects in randomized clinical trials of granulocyte transfusion therapy is that granulocytes are offered at many institutions as part of standard of care despite there being no definitive evidence of clinical efficacy. This practice coupled with the possibility that subjects may be randomized to the non-granulocyte transfusion group serves as a disincentive for clinicians who believe that granulocyte transfusion therapy offers a benefit from enrolling their patients in a randomized-controlled clinical trial (19). Without a sufficient number of subjects in randomized-controlled clinical trials, it will not be possible to have adequate power to detect any difference between a granulocyte treated group and control group.

In an effort to take advantage of the standard of care treatment of neutropenic patients with granulocytes, an online registry, PROspective GRanulocyte usage and outcomEs Survey (ProGrES) was created to gather information on granulocyte transfusion practices from different countries around the world (5). The goal of the registry is to characterize current practices and describe outcomes. Prospective data is being collected at various times points; namely, time of granulocyte request, weekly, 28 days and 6 months. Information that is gathered includes donor identifier, granulocyte unit, patient characteristics, illness characteristics, and outcomes. This registry provides a platform to explore the relationship between intervention and outcomes and will hopefully generate enough evidence to support or refute the efficacy of granulocyte transfusion therapy (5).

Conclusions

Granulocytes are our natural defenders against bacterial and fungal invaders. Patients undergoing chemotherapy develop severe neutropenia and become susceptible to severe bacterial and fungal infections which lead to significant morbidity and even mortality. Granulocyte transfusion therapy became possible when the technology that allows the collection of granulocytes from a donor's peripheral blood circulation was developed. Decades after granulocyte transfusion therapy first began, there are still no definitive evidence-based practice guidelines. Until sufficient numbers of subjects can be enrolled in randomized-controlled clinical trials and an adequate number of high-dose granulocyte products can be obtained consistently for these trials, the conclusive demonstration of clinical efficacy for granulocyte transfusion therapy may remain elusive.

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Footnote

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