

Granulocyte Transfusion in the G-CSF Era

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Abstract

Granulocyte transfusions have been used since the 1960s with varying degrees of clinical success in the treatment of infection in patients with neutropenia or inherited granulocyte disorders. A number of studies have indicated that efficacy may well be associated with the dose of granulocytes delivered. Collection of granulocytes using modern apheresis machines and corticosteroid administration yields approximately $20\sim 30\times 10^9$ neutrophils, unlikely to be adequate for treating an established infection. The administration of G-CSF to healthy donors has resulted in average granulocyte yields up to 8×10^{10} cells. Normal or near normal blood neutrophil counts are often attained when these concentrates are transfused to neutropenic recipients, and these levels are sustained for up to 24 h. G-CSF-primed granulocytes appear to be functionally normal by both in vitro and in vivo measurements. Adverse effects experienced by recipients are similar to those seen with traditional doses of granulocytes. G-CSF administration to donors is well tolerated. Controlled clinical trials are needed to determine the therapeutic efficacy of G-CSF-primed granulocyte transfusions.

Neutropenia is a major risk factor for the development of severe bacterial and fungal infection in patients undergoing hematopoietic stem cell transplantation or aggressive chemotherapy for malignancy [1-3]. Despite the use of modern antibiotics and growth factors, infection remains the major cause of morbidity and mortality in these patients. The 5-year incidence of pulmonary aspergillosis infection in patients undergoing bone marrow transplantation in the era of fluconazole prophylaxis now approaches 13%; approximately 25% of these infections occur during therapy-induced neutropenia. Mortality with these mold infections has traditionally been 65~85% [4-6]. The strongest predictor of progression or recovery from invasive fungal infection in the cancer/marrow transplant setting is the recovery of adequate neutrophils [7].

Neutrophil transfusion therapy is a logical approach to this problem and has been available to clinicians for almost 30 years. Promising results from early uncontrolled trials [8-10] led to great enthusiasm for this therapy. However, in spite of the fact that some degree of clinical utility was subsequently demonstrated in five of seven controlled trials [11], granulocyte transfusion therapy fell into disuse in the 1980s and 1990s, largely because the results were not impressive to clinicians. A

likely important explanation for these inconsistent results was that inadequate doses of neutrophils were being provided. In addition, granulocytes rapidly undergo apoptosis after collection, limiting their ex vivo shelf life and their in vivo survival.

The importance of the granulocyte dose was suggested by the early uncontrolled trials; patients who received higher doses appeared to have a higher rate of response [8,9]. Retrospective analysis of the seven controlled trials demonstrated that one of the important factors determining success was the dose of granulocytes delivered [11,12]. Finally, a number of animal studies have shown that granulocyte dose was directly related to survival in a sepsis model [13] and to the ability of granulocytes to migrate to the cerebrospinal fluid in a meningitis model [14]. These findings are not surprising in view of the fact that traditional doses of granulocytes did not exceed $20\sim 30\times 10^9$ cells, about half of expected daily marrow production in a normal non-infected adult [15]. With the availability of G-CSF it became possible to examine the possibility that much larger doses could be obtained by G-CSF stimulation of the donors.

1. Use of G-CSF to Stimulate Granulocyte Donors

G-CSF, an 18~22 kd glycoprotein, stimulates marrow granulocyte proliferation, causes the release of granulocytes into the bloodstream, and appears to be the principal regulator of normal granulocytopoiesis [16,17]. Two recombinant forms are available filgrastim is a non-glycosylated form and lenograstim is a glycosylated form. Several studies using G-CSF to stimulate healthy granulocyte donors have now been performed [18-28]. These studies consistently show that the administration of G-CSF results in mean granulocyte yields as high as 82×10^9 , values that are two-to-four times higher than that achievable with corticosteroid priming alone. The highest yields are obtained by administering both G-CSF and dexamethasone approximately 12 h before the collection [20,22,27]. The function of neutrophils obtained from donors who have been treated with G-CSF, with or without additional corticosteroids, appears to be normal or near normal when measured in vitro. Dale et al showed that such cells exhibited normal behavior in chemiluminescence and bactericidal assays. Some studies also suggest that G-CSF augments the antifungal activity of granulocytes for species that include *Aspergillus* and *Rhizopus* [29,30]. When these cells were reinfused into the donor, intravascular survival was prolonged, and the cells were capable of migrating to extravascular sites [31]. Studies by Adkins et al [32] have shown that ^{111}In -labeled neutrophils collected from G-CSF stimulated donors are capable of localizing to inflammatory sites in the transfusion recipients. Similarly, Price et al measured buccal neutrophil accumulation in neutropenic recipients and showed that neutrophils collected from G-CSF stimulated donors are capable of extravascular migration [21].

2. Hematologic Effects in Recipients

In most reported studies, transfusion of neutrophils from G-CSF stimulated donors results in large post-transfusion neutrophil increments, sometimes resulting in normal or near normal neutrophil counts. The increase in neutrophil count is often sustained and maintained above baseline for 24 hours or more. This response is in marked contrast to the experience with lower doses of neutrophils in which post-transfusion increments have been small, if any, and the increase sustained for a few hours at most. Hester et al [33] reported a mean post-transfusion increment of $0.6 \times 10^3/\mu\text{l}$ after transfusion of 41×10^9 neutrophils. The neutrophil count was still substantially above baseline 24 hours later. Adkins [34] delivered a slightly higher dose of cells (51×10^9 neutrophils) and observed a post-transfusion increment of $1 \times 10^3/\mu\text{l}$, a count which was sustained for 1~1.5 days. Price et al [21] transfused an average of 82×10^9 neutrophils and observed a post-transfusion increment of $2.6 \times 10^3/\mu\text{l}$ with next morning neutrophil count of $2.6 \times 10^3/\mu\text{l}$. In the study of Price et al [21], where the highest dose was provided, the average neutrophil count

after transfusion was in the normal range. The prolonged survival of these transfused cells in the circulation essentially means that most severely neutropenic patients, given a large enough dose and transfused daily, can be converted to patients with sustained normal or near normal neutrophil counts. The reason for the prolonged survival of these transfused cells is not known but may be related to a number of factors, including the shift of relatively young cells into the donor's circulation from the marrow, the alterations typically seen in the expression of surface adhesion molecules, and the anti-apoptotic effect of G-CSF. Finally, the transfused cells appear to migrate to inflammatory sites normally [21].

3. Clinical Efficacy of G-CSF-stimulated Granulocyte Transfusions

The evidence that provision of granulocytes from G-CSF stimulated donors is efficacious in clearing infection or prolonging patient survival is limited to that provided by case reports [35-39] or small uncontrolled series [25,33,40,41]. Hester et al treated 15 patients with established fungal infection and reported that 60% responded to transfusion [33]. Taylor et al reported that 15/18 patients with either bacterial or fungal infection recovered after a series of granulocyte transfusions [41]. Less encouraging results were obtained in the eight patients treated by Grigg et al [25], although all three patients with bacterial infection recovered, all five of the patients with fungal infection died. Price et al administered granulocytes to nineteen bone marrow transplantation patients with bacterial or fungal infection; infection resolved in eight of eleven patients with bacterial or candidal infection [21].

4. Adverse Effects in Recipients

G-CSF-primed granulocyte transfusions are tolerated relatively well by recipients, with adverse reactions seen in 6~13% of transfusions [21,23,42]. The most frequent reactions are fevers and chills. Mild hypoxemia (<6% change in O_2 saturation) and shortness of breath may be seen in up to 7% of transfusions, with a much smaller proportion of transfusions leading to severe hypoxia. These figures do not appear to be different from the incidence of adverse effects seen in non-G-CSF-stimulated granulocyte transfusions. In particular, pulmonary complications occur with a similar frequency, despite the larger dose of granulocytes given [43]. In studies performed in bone marrow transplant patients, the presence of human leukocyte antigen (HLA) antibodies in the recipient, or of a positive HLA-crossmatch between donor and recipient, does not appear to correlate with the occurrence of adverse reactions [21,23].

5. Adverse Effects in Donors

Short term administration of G-CSF to normal donors is generally well tolerated. Stroncek et al [44] and

Anderlini et al [45] have reported on the effects of five days of G-CSF in 142 donors. Symptoms were noted in 90~98% of donors. Bone aching, headache, myalgias, fatigue, and nausea were seen in approximately 80%, 40~70%, 25%, 15%, and 10% of subjects, respectively. In most subjects, the symptoms are mild to moderate, and less than 10% experience symptoms so severe that the drug has to be discontinued or the dose modified. Symptoms resolve within a few days after the drug is discontinued, and the donor's leukocyte count returns to normal within 7~10 days. Adverse effects in donors stimulated with only one dose of G-CSF are similar to those in donors given multiple doses, but the incidence is less, the symptoms are not as severe, and the duration is shorter [18,20,21,31,46]. In a recent study of 175 granulocyte collections, 41%, 30%, and 30% of donors stimulated once with 600 g G-CSF and 8 mg dexamethasone experienced mild to moderate bone pain, headache, and insomnia, respectively. Twenty eight percent of these donors experienced no side effects, and 98% indicated a willingness to undergo future G-CSF stimulation for leukapheresis [21].

6. Summary

Numerous studies have suggested that the success of granulocyte transfusion therapy is likely to be dependent on the dose of granulocytes provided. The use of G-CSF to stimulate granulocyte donors causes marked neutrophilia in these donors and results in greatly increased granulocyte yields. Transfusion of these cells into neutropenic recipients can result in normal or near normal blood neutrophil counts and migration of the transfused cells to inflammatory sites. Although some results to date are encouraging, there are currently inadequate data to determine the effect on clearance of infection or patient survival. Controlled trials are needed to determine whether granulocyte transfusion therapy using these large doses of cells is efficacious and cost effective.

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