Granulocyte transfusion

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Purpose of review

Granulocyte transfusions have been used for more than four decades. Several issues have complicated the analysis of previous studies, including the utilization of improved antimicrobials, the effects of recipient alloimmunization and variable cell dose. The use of granulocyte colony-stimulating factor for donor stimulation has revived interest in granulocyte transfusion. The aim of this review is to evaluate the most recent studies in granulocyte transfusion therapy and their clinical applicability.

Recent findings

Granulocyte colony-stimulating factor use has increased the granulocyte yield by approximately fourfold. Multiple recent studies have shown that granulocyte transfusions can be helpful in controlling severe infections progressing despite the use of appropriate antibiotics, with a response rate of 40–80% with variability in results depending on patient characteristics. This benefit is limited to a small patient population as the incidence of prolonged reversible neutropenia is relatively small. Severe side effects have been rare in those studies.

Summary

Granulocyte transfusions are beneficial in neutropenic patients with severe uncontrolled infection. The underlying disease process is the major determinant of outcome in these patients. Because granulocyte transfusions are not commonly used, centers are not currently able to provide transfusions in a timely fashion. Nonalloimmunized patients can receive cells from nonmatched ABO compatible donors, while alloimmunized patients should receive granulocytes from either HLA-matched donors or donors selected by leukoagglutination or lymphocytotoxicity crossmatching. Further studies are needed to clarify the optimal starting time and frequency of transfusions, and the best method for identifying donor – recipient compatibility.

Keywords

granulocytes, neutropenia, transfusion

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Abbreviations

CML	chronic myeloid leukemia
G-CSF	granulocyte colony-stimulating factor
GVHD	graft-versus-host disease

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Introduction

The use of granulocyte transfusions has varied in the past four decades. Their use was maximal in the 1970s but gradually decreased in the 1980s, with increasing data suggesting that it had minimal impact on outcome. There has been a recent reemergence in their use since the availabilty of granulocyte colony-stimulating factor (G-CSF), as priming of donors has enabled the procurement of greatly increased numbers of granulocytes for transfusion. In this review we will provide a historical perspective and discuss indications for granulocyte transfusions, collection methodology and the results of recent clinical trials using G-CSF mobilized transfusions.

Historical background

The relationship between the degree and duration of neutropenia and the risk of infection was first reported in the 1960s [1]. As the use of chemotherapy for the treatment of malignancy rapidly increased, the incidence of neutropenia and infection increased. This led to the concept of granulocyte replacement for the treatment of infections. Granulocyte concentrates were initially obtained from chronic myeloid leukemia (CML) patients, with a high white blood cell count, by the crude technique of whole blood sedimentation [2]. This process was limited by donor availability and ABO compatibility. Nevertheless, two very important principles were recognized: the correlation of cell dose with response; and the observation that transfusion reactions were increased and efficacy decreased in alloimmunized patients.

With the development and increased availability of centrifugation blood cell separators, the use of granulocyte transfusions increased. Granulocytes were collected from normal healthy donors, but cell dose was usually insufficient. Filtration techniques, which are now only of historical interest, were then developed. In brief, heparinized blood was passed through a nylon fiber filter, and the granulocytes would then be trapped and collected off the filter. Filtration yielded higher granulocyte collections, but the process of adhesion and elution led to activation of the granulocytes and more transfusion

reactions in the recipient. This procedure was abandoned with further improvement in centrifugation technology [3].

A series of randomized controlled clinical trials of granulocyte transfusion were conducted and published in the period between 1972 and 1977 [4–9]. All but one study [9] showed benefit for recipients of granulocyte transfusion, especially for patients with prolonged neutropenia. These studies used filtration [5,7], centrifugation [6,9], or both for collection [4,8] of granulocytes from healthy donors. In three of those studies [5–7], the donors were given corticosteroids to increase their granulocyte count and hence increase the granulocyte yield. The granulocyte yield by centrifugation alone, filtration alone, centrifugation with corticosteroids, and filtration with corticosteroids was less than 10^{10}, 2×10^{10}, 2.6×10^{10} and 5.9×10^{10}, respectively. All but one study [7] evaluated the recipients for the presence of antibodies against the donor granulocytes either by leukoagglutination or lymphocytotoxicity. These studies would be faulted by current standards. The number of patients was small, response definitions were vague and not all patients had documented infections.

The use of granulocyte transfusions then diminished, probably because clinicians were seeing severe reactions with less clear benefit. Furthermore, the need for transfused granulocytes seemed less pressing as antimicrobials rapidly improved. In addition, the only randomized controlled study of granulocyte transfusions in the 1980s, a study by Winston *et al.* in 1982 [10], showed no benefit for granulocyte transfusion. In this study patients with documented infections and resistance to antibiotic therapy were randomized to continued antimicrobial therapy either with or without granulocytes collected by centrifugation. The median cell dose was low (5.9×10^9), however, and testing for histocompatibility was not done as in the earlier studies.

Alloimunization

The effect of alloimmunization on the efficacy of granulocyte transfusion was first studied and reported in animals in the early 1970s. Granulocyte increment and migration to tissues were lower in recipient beagles that were sensitized to donor foxhound antigens [11].

In a small study by Dahlke *et al.* [12], 31% of patients who had a poor outcome with granulocyte transfusion had granulocyte antibodies detected by the granulocyte indirect immunofluorescence test (GIIFT), in contrast to only 4% of patients who had a favorable outcome, but there was no relation to the presence of lymphocytotoxic antibodies. Two groups of investigators used ^{111}In labeled granulocytes to assess the role of alloimmunization on granulocyte migration [13,14]. A positive leukoagglutinin crossmatch was a good predictor of poor migration in both studies, but the effect of a positive lymphocytotoxic response was predictive in only one of the studies [13]. In the study by Dutcher *et al.* [13] platelet refractoriness was associated with poor granulocyte migration, suggesting that this may be used as a clinical surrogate for the presence of granulocyte alloimmunization. Based on the post granulocyte transfusion increment, Ungerleider *et al.* [15] reported that histocompatibility did not affect outcome. In that study, the magnitude of granulocyte rise was too small to detect any difference in outcome. In another study, the screening lymphocytotoxic assay was the best predictor of response, while no relationship was found between the baseline recipient crossmatch lymphocytotoxic assay, leukoagglutination assay, donor–recipient HLA disparity and response [16]. In short, the best method to accurately assess donor and leukocyte compatibility is yet to be determined.

Granulocyte colony-stimulating factor

The availability of myeloid colony stimulating factors renewed the interest in granulocyte transfusion. The use of G-CSF was studied by Bensinger *et al.* [17], who established its safety and efficacy in normal granulocyte donors. Normal humans produce about 10^{11} granulocytes daily, with the ability to markedly increase that during stress [18]. With intermittent flow centrifugation technology from donors premedicated with corticosteroids only, the maximum number of cells transfused would usually not exceed 10^{10}. Currently, with the use of continuous flow centrifugation and donors premedicated with G-CSF with or without corticosteroids, the yields are 4×10^{10} or more. Furthermore, the use of G-CSF has led to sustained posttransfusion granulocyte increments, perhaps secondary to the mobilization of early precursors which continue to proliferate in the recipient. This phenomenon has previously been described in recipients of transfusions from CML patients [19], in which seven out of 14 patients had a granulocyte count over 500/μl for four or more days after the last white blood cell transfusion. In addition, granulocytes obtained from normal donors by G-CSF stimulation and centrifugation have been found to have normal function both *in vivo* and *in vitro* [20].

Several trials [21–23,24•,25,26] have investigated the efficacy of granulocyte transfusion from G-CSF stimulated donors with or without corticosteroids. The addition of corticosteroids increased the granulocyte yield by two to threefold. All studies were phase I or II trials, and no randomized controlled trial has been published in the last 20 years. Most of these studies ignored the earlier observations about alloimmunization, but the median cell dose was usually adequate ($>2 \times 10^{10}$). The timing of granulocyte transfusion ranged from a median of 2 to 13 days after the beginning of antibiotics. Response rates

of 40–80% were observed in patients with severe uncontrolled infections, but the overall survival was determined by the underlying disease process and endogenous neutrophil recovery. In a recent study [24], 84% of patients who had an infection that was progressively worse after 48 h of appropriate antimicrobial therapy had resolution of the infection with the use of granulocyte transfusions. All donor samples were lymphocytotoxic crossmatched with the recipient. Unfortunately, the median survival for this group was 170 days with a projected 3-year survival of 5%. The patients succumbed to disease progression, graft-versus-host disease (GVHD), continuing aplasia and other causes, emphasizing that the underlying disease process was the major predictor of survival. Bacterial infections have consistently responded better than fungal infections [23,25], with a suggestion in one study that Gram-negative infections respond better than Gram-positive infections [23]. It is difficult to compare these studies because of differing definitions of response, variation in the fraction of patients with marrow recovery and nonstandardization of the types and severity of infections. Hubel *et al*. [27] assessed the efficacy of granulocyte transfusions from related versus unrelated donors and compared them to matched controls in allogeneic stem cell transplant patients. There was no difference in the response rates, absolute neutrophill count (ANC) increments, adverse effects and the rate of acute GVHD between the two granulocyte transfusion groups. The incidence of grade IV acute GVHD, however, was statistically higher in patients with unrelated donors compared with matched controls (23% versus 3%). The authors concluded that the correlation was difficult to ascertain as the patient numbers were small. Surprisingly there was a longer delay between requesting and obtaining transfusions in the related versus unrelated donors. Although response rates in some studies have been low, it should be kept in mind that those infections were uncontrolled and that the mortality rate would have probably been much higher with conventional therapy.

Prophylactic granulocyte transfusion

Granulocyte transfusions have been used to prevent infections (primary prophylaxis) and to prevent reactivation of infections (secondary prophylaxis) during periods of prolonged neutropenia. The role of primary prophylactic granulocyte transfusion in patients with expected prolonged neutropenia has been addressed in several trials and has been reviewed in a previously published metaanalysis [28]. Overall, there has been no definite benefit for this approach. In addition, the risks outweigh the benefits for this procedure due to the increased incidence of alloimunization, the development of lymphocytotoxic antibodies and platelet refractoriness [29]. Granulocyte transfusions have been used as secondary prophylaxis against fungal infections [24•,30] and none of the patients in these two series had reactivation of their infections. Other studies using voriconazole alone, however, have been equally successful [31].

Therapy for invasive fungal infections

Severe uncontrolled fungal infections in neutropenic patients are likely to provide the major target of this technology. Half of neutropenic patients with invasive aspergillus have progressive infection despite aggressive antifungal therapy [32]. In neutropenic patients with refractory fungal infections, granulocyte transfusions have shown a favorable outcome in 35–78% [22,24•, 25,27,33] of patients. Two of the studies with the highest response rates used either HLA-matched donors [33] or screened donors with the lymphocytotoxic assay [24•], emphasizing the importance of donor/recipient leukocyte compatibility. In the study by Dignani *et al*. [33], 11 of 15 evaluable patients with neutropenia-related, amphotericin-resistant fungal infections had a favorable response. Histocompatibility was not assessed, and starting granulocyte transfusions earlier yielded a better outcome. In a single institution retrospective analysis of 158 leukemia patients with candidal infections, 25 patients received granulocyte transfusions. Mortality was lower in the group that received granulocyte transfusion, although they had had multiple predictors of increased mortality [34•]. Most studies have avoided the simultaneous administration of amphotericin B and granulocyte transfusions within a short period of time as there has been a reported increased risk of adverse reactions by some [35], but not all investigators [36]. We generally recommend separating the administration of granulocyte transfusion and amphotericin B by 6–8 h. Practically, this is best accomplished by giving the amphotericin B in the morning while the granulocytes are being collected.

Donor side effects

In a prospective trial, healthy donors who received G-CSF with or without dexamethasone as compared with dexamethasone alone had more side effects in the form of bone pain, headache, moderate insomnia and nausea. All side effects were transient, although 5% of donors labeled the bone aches as intolerable pain. In addition, itching was observed in a small number of donors, most probably secondary to hydroxyethyl starch (HES). The median cell dose in collections following dexamethasone alone, G-CSF alone and both were 1.3, 5.6 and 8.3×10^{10}/U, respectively. There has been one case series reporting increased incidence of cataracts in frequent donors [37], and currently there is an ongoing clinical trial collecting data on granulocyte donors who have received repeated doses of corticosteroids for granulocyte mobilization.

Recipient side effects

Perhaps one of the major factors which led to the diminution of granulocyte transfusions was the concern

regarding side effects. With current centrifugation techniques, most studies have reported a low incidence of side effects. The incidence of infusion-related side effects of granulocyte transfusion ranges from 5 to 13% [25,33], with only approximately 0.1% being hypoxemia with bronchospasm. Granulocyte transfusion should be given slowly, and premedication with acetaminophen and benadryl is recommended. A negative CMV donor serology is required in CMV-negative recipients. CMV-negative recipients of CMV-positive allogeneic stem cell transplantation may receive CMV-positive granulocyte transfusions without an increased risk of infection [38]. Long-term side effects, especially in patients requiring repeated transfusions, include the development of alloimmunization and platelet refractoriness [29].

Conclusion

Granulocyte transfusion has a definite but limited role in the treatment of neutropenic patients. The underlying disease process and neutrophil recovery are still the main determinants of outcome in neutropenic patients with severe infections. The patient population that would benefit from this therapeutic modality is very limited since both G-CSF and peripheral blood stem cell transplantation have decreased the duration of neutropenia. In addition, only patients with reversible neutropenia, with a severe uncontrolled infection, who are not expected to recover their counts in approximately 5 days would benefit from this therapeutic modality. It is often helpful to obtain a bone marrow biopsy before starting granulocyte transfusions to better predict the expected granulocyte recovery time. If possible, recipients should be screened for the presence of donor antileukocyte antibodies, but the best method remains to be elucidated by further investigations. A satisfactory response to platelet transfusion is a reasonable clinical surrogate, suggesting an absence of significant recipient alloimmunization. Alloimmunized patients should receive HLA-matched granulocyte transfusions as they are at higher risk for developing severe reactions and are not likely to benefit if they receive non-HLA matched transfusions. Most studies used irradiated granulocyte concentrates. The possible increased risk of acute GVHD in allogeneic stem cell transplant patients must be weighed against the benefit and requires additional clarification. Donors may be mobilized with G-CSF 5 μg/kg the day before donation followed by additional doses if subsequent donations are required. Donors may be either family members or random donors. Daily granulocyte transfusions should continue to maintain a granulocyte count of more than 500/μl until neutrophil recovery, clinical improvement or stability. Further studies are required to better define the optimal starting time and frequency of granulocyte transfusions.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 59).

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