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# The role of granulocyte transfusions in neutropenic patients

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## Summary

The precise role for donor granulocyte infusions remains to be delineated, partly because of the lack of defined clinical trials. The aim of this article is to summarize the studies undertaken so far and highlight the logistical problems associated with undertaking future studies. We also aim to provide a practical guide to the application of this therapeutic approach.

**Keywords:** granulocyte, transfusions, neutropenia, granulocyte colony-stimulating factor.

## Historical perspective

Ever since Metchinikoff described phagocytic white cells or neutrophils in 1883 (Metchinikoff, 1967), the primacy of their role in fighting infectious disease has never been questioned. Indeed, Strumia (1934) injected neutrophils intramuscularly into neutropenic patients in the hope that breakdown products would stimulate endogenous neutrophil function. It is needless to say that these experiments were less than successful. Subsequently Brecher *et al* (1953) undertook experiments in dogs, which indicated that harvested neutrophils could circulate and migrate to sites of inflammation. The quantitative relationship between circulating leucocytes and infection in patients with acute leukaemia, and the potential for leucocyte transfusion in the management of neutropenic patients was established in the 1960s (Freireich *et al*, 1965; Levin *et al*, 1965; Bodey *et al*, 1966).

## Justification for granulocyte transfusions

For patients undergoing stem cell transplantation or induction for acute leukaemia, modern intensive chemotherapy often results in frequent and prolonged periods of neutropenia, a major risk factor for severe bacterial and fungal infection (Pizzo, 1993; Hughes *et al*, 1997). Despite the use of broad-spectrum antibiotics and colony-stimulating factors, infection can account for 40% of deaths in these patients (Bodey *et al*, 1992), and the incidence appears to depend upon the degree and duration of neutropenia and immunosuppression. The early use of broad spectrum antibiotics (prophylactic and empirical) has resulted in a significant improvement in outcomes for patients developing bacterial infections, but shifts in the emerging pattern of pathogens, antibiotic resistance and changes in host immune patterns leave no room for complacency and will pose potential management problems in the future. The development of vancomycin-resistant *Enterococcus* and methicillin-resistant *Staphylococcus aureus* is a reminder of the potentially devastating consequences of bacterial infections. Furthermore, fungal and viral infections are increasingly a major problem in haematology patients and now represent a major cause of morbidity and mortality (Chopra, 2002; Hassan *et al*, 2003).

Infectious risk can be minimized with scrupulous hygiene measures, use of laminar airflow or high efficiency particle air filtration systems, and the use of high yield diagnostic strategies preventing treatment delay. Once invasive fungal infection is present, the best predictor of recovery is for the return of endogenous neutrophil production (Vassilev *et al*, 1999), and in this situation colony-stimulating factors have only had a limited impact. The rationale for granulocyte transfusion therefore remains compelling.

The half-life of the neutrophil is approximately 7 h. In order to raise the circulating granulocyte count from zero to the normal range in an average 70 kg individual would require the infusion of $40–60 \times 10^9$ cells/d. In earlier studies, patients with high circulating white cell counts secondary to chronic myeloid leukaemia were sought as donors, but the paucity of such individuals was a major barrier to systematic studies. The introduction of cell separator and leucocyte filtration machines together with the use of steroids to mobilize neutrophils to the peripheral circulation had the potential to apply just this type of replacement therapy (Young, 1981).

A number of clinical questions however remains to be addressed. These included the efficacy of granulocyte transfusions, clinical indications for transfusions and the justification for prophylactic transfusion. Furthermore, the source of granulocytes, the cytomegalovirus (CMV) and human leucocyte antigen (HLA) status of potential donors was investigated for their effect on the replacement. As will be clear from this annotation, these questions remain to be fully addressed to this

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day. The main problem with early and more recent studies has been the lack of consistency in selecting appropriate patients for granulocyte transfusions and the selection of appropriate controls. More importantly, given the fact that 60–80% of neutropenic patients will recover from neutropenic sepsis without bone marrow reconstitution but with appropriate broad-spectrum antibiotics, the additional benefit of infused granulocytes will require trials with large numbers of patients to obtain the statistical power to indicate benefit. Early trials also suffered from the fact that patients were only evaluated for short-term survival and often patients succumbed from their haemorrhage or leukaemia progression.

## Evaluation of early clinical studies

Early uncontrolled trials with glucocorticoid stimulation gave promising results and seven subsequent controlled trials between 1972 and 1982 assessed the efficacy of granulocyte transfusion in neutropenic adults with clinical evidence of infection (Graw *et al*, 1972; Fortuny *et al*, 1975; Higby *et al*, 1975; Alavi *et al*, 1977; Herzig *et al*, 1977; Vogler & Winton, 1977; Winston *et al*, 1982). Three studies showed clear benefit, two showed benefit in certain patients, and two were negative (Table I). All of these studies involved small numbers of patients. It should also be noted that patients who received a white blood cell transfusion might be a selected, better-prognostic group, in that they survive long enough to receive transfusion, and possibly achieve marrow recovery.

In a review of granulocyte infusions conducted over a 1-year period, increased granulocyte dose and leucocyte compatibility were identified as important factors in positive clinical outcomes (Strauss, 1993). Additionally, the granulocyte dose was demonstrated to be directly related to survival in a sepsis model (Appelbaum *et al*, 1978). However, more recent studies emphasized the importance of the subsequent neutrophil increment after transfusion over the granulocyte cell number infused.

The role of prophylactic granulocyte transfusions was equally controversial. Clift *et al* (1978) undertook a randomized trial in 69 patients receiving a bone marrow transplant (BMT) for haematological malignancy or aplastic anaemia. Patients were randomized to receive granulocyte infusions when their circulating neutrophil count fell below $0{\cdot}2 \times 10^9$/l between the post-transplant period and engraftment. In the first 21 d post-transplant there were two local infections and no septicaemic episodes in the 29 transfused patients. Seven local and 10 septicaemic episodes occurred in the 40 control patients. More than 40% of patients also received broad spectrum antibiotics at the time when the granulocytes were transfused and it is difficult to ascertain the relative contribution of the antibiotics as opposed to the cells infused. A study from the University of California, Los Angeles (UCLA) in afebrile BMT patients who had not received antibiotics on admission showed no conclusive trend to the efficacy of granulocyte transfusions (Winston *et al*, 1980). Therefore, granulocyte transfusion became a seldom-used technique in the 1980s and early 1990s because of lack of perceived observable benefit. Traditional doses of granulocytes stimulated with dexamethasone or prednisolone did not exceed $20–30 \times 10^9$ cells, around half of the expected daily marrow production in the non-infected patient. The availability of haematopoietic growth factors, particularly granulocyte colony-stimulating factor (G-CSF) has raised the possibility of stimulating normal donors, allowing harvesting of larger cell doses.

## Analysis of G-CSF-mobilized granulocytes and evaluation of recent studies

G-CSF is an 18–22 kd glycoprotein that mobilizes granulocytes into peripheral blood from bone marrow, allowing apheresis to collect larger cell numbers. In normal donors the response to G-CSF is dose-dependent with an increase in the neutrophil count within 2 h, peaking at 12 h. Granulocyte-macrophage colony-stimulating factor (GM-CSF) also has a dose-dependent effect on granulocyte and monocyte mobilization along with an effect on magakaryocyte and erythroid precursor populations, which appears to be negligible *in vivo*. Several studies have looked at the optimization of dosing, with G-CSF dose ranging from 5–10 μg/kg, and optimal yields appear to be obtained by administering G-CSF with 8–12 mg of dexamethasone or 60 mg of prednisolone approximately 12 h prior to

**Table I.** Results of early controlled studies evaluating granulocyte transfusions in neutropenic sepsis.

| | Study group | | | Control group | | | |
|---|---|---|---|---|---|---|---|
| Study | No. of Patients | Survived | % | No. of Patients | Survived | % | Success |
| Higby *et al* (1975) | 17 | 13 | 76 | 19 | 5 | 26 | Yes |
| Vogler and Winton (1977) | 17 | 10 | 59 | 13 | 2 | 15 | Yes |
| Herzig *et al* (1977) | 13 | 10 | 75 | 14 | 5 | 36 | Yes |
| Alavi *et al* (1977) | 12 | 10 | 82 | 19 | 12 | 62 | Partial |
| Graw *et al* (1972) | 39 | 18 | 46 | 37 | 11 | 30 | Partial |
| Winston *et al* (1982) | 48 | 30 | 63 | 47 | 34 | 72 | No |
| Fortuny *et al* (1975) | 17 | 13 | 78 | 22 | 18 | 80 | No |

collection (Liles *et al*, 2000; Stroncek *et al*, 2001). Average cell yields mobilized in this manner range 50–70 $\times$ $10^9$ neutrophils; much greater than previously possible and enabling the restoration of normal neutrophil levels.

Continuous flow centrifugation leukapheresis is performed, rather than filtration leukapheresis, which damages or activates cells with granule release and complement activation (Liles *et al*, 2000; Stroncek *et al*, 2001), and approximately 7 l of blood is processed in approximately 3 h. Granulocytes then have to be separated from red blood cells by a hydroxethyl starch sedimenting agent. Pentastarch has a rapid elimination time but collections are lower, and hetastarch is more effective but persists in the circulation. After processing, the granulocytes are suspended in 400 ml of plasma along with a relatively high red blood cell and platelet content. They can be stored for up to 24 h at room temperature without agitation but progressive loss of function occurs with storage and therefore they are usually transfused as soon as possible after collection. It has been shown that bactericidal (*S. aureus*) and fungicidal (*A. fumigatus R. arrhizus C. albicans*) abilities can be maintained in cells over a 48-h storage period at 10°C (Hubel *et al*, 2000).

Granulocytes are transfused daily and at least 4-d treatment is recommended, with continuation after this time based upon clinical judgement and patient response. The high red cell content of the product means that donors must be ABO compatible, while the presence of viable lymphocytes means that severely immunosuppressed patients are at risk of graft-*versus*-host disease. Most studies suggest that irradiation does not affect neutrophil function, and so products to be used in these patients should be irradiated (Haidenberger *et al*, 2003). Granulocytes carry the same risk of infectious disease transmission as other blood products, and the frequent need for transfusion prior to completion of viral testing means donors need to be carefully selected. CMV-negative products should be given to CMV-negative patients (see below). It should be noted that an analysis using the Cochrane database (Mohan & Brocklehurst, 2003) did not identify any reports of transmission of viruses. There are, however, no formal studies to assess safety, but viral transmission is possible, hence the requirement for testing for hepatitis B and C viruses and human immunodeficiency virus. It should also be noted that granulocytes for transfusion are irradiated and therefore lymphotropic viruses, such as human T-cell lymphotrophic virus type I, Epstein–Barr virus and human herpesvirus 6 are less likely to be transmitted. This, however, is not the case for organisms such as Toxoplasma. Indeed, reports of toxoplasmosis have been published (Siegel *et al*, 1971).

The feasibility of using community donors and related donors has been assessed, and showed that patients who received granulocyte concentrates from community donors had less delay from the time of infection to transfusion and also significantly higher absolute neutrophil increments. Clinical outcome appears to be comparable between the groups after 6 months, suggesting community donors can be used for the early institution of granulocyte transfusion therapy (Price *et al*, 2000; Hubel *et al*, 2002).

Neutrophil function following G-CSF has been an area of interest, and it would appear that these neutrophils exhibit normal behaviour in bactericidal assays with a maintained inducible respiratory burst *in vitro*. *In vivo*, the buccal neutrophil response, a measure of the neutrophil capacity to migrate to tissue sites, is restored to normal with granulocyte transfusion (Price *et al*, 2000), while indium-labelled granulocytes were shown to localize to inflammatory sites in recipients in the same way as normal cells (Adkins *et al*, 1997). Indeed, in the study by Adkins *et al* (1997), two patients showed neutrophil localization in the oropharynx during mucositis episodes, while another demonstrated localization in the ileum during episodes of diarrhoea. Immunophenotypic changes in neutrophils in normal donor subjects after G-CSF show increased expression of HLA-DR, CD11b, CD14, CD18, CD32, CD34, CD64 and CD71, with decreased expression of CD10, CD15 and CD16 (Zarco *et al*, 1999; Hubel *et al*, 2000). These markers imply immaturity except for CD14, which is responsible for the binding of neutrophils to lipopolysaccarides, suggesting possible increased functional activity against Gram-negative organisms. Phenotypic changes in donors parallel blood G-CSF levels and return to normal 1 month after G-CSF treatment. It should be noted that recipients of granulocyte transfusion will also be receiving G-CSF and this may maintain this possible increased functional activity, as well as improving circulation, survival, and increment levels.

Newer studies have shown preliminary evidence that suggest G-CSF-stimulated granulocytes may be a safe therapeutic measure with beneficial effects in neutropenic patients with serious infections. The literature however is confined to small, uncontrolled series and case reports; there are no well-designed trials with clinically relevant endpoints (Hester *et al*, 1995; Taylor, 1996; Peters *et al*, 1999; Price *et al*, 2000; Lee *et al*, 2001; Cesaro *et al*, 2003). The outcomes of the studies in patients with established infections are indicated in Table II. All patients had progressed despite broad spectrum anti-infective drugs and a significant proportion of patients had fungal infections.

**Table II.** Recent studies evaluating granulocyte transfusion in prolonged refractory neutropenic sepsis.

| Study | No. of Patients | No. responded | % Response |
|---|---|---|---|
| Lee *et al* (2001) | 25 | 10 (4/16 B, 6/9 F) | 40 |
| Cesaro *et al* (2003) | 15 | 6 CR, 3 PR | 60 |
| Hester *et al* (1995) | 15 | 9 | 60 |
| Peters *et al* (1999) | 30 | 19 (14/17 B, 5/13 F) | 63 |
| Price *et al* (2000) | 16 | 8 (8/11 C + B, 0/5 A) | 50 |
| Taylor (1996) | 18 | 15 | 83 |

CR, complete response; PR, partial response; B, bacterial; F, fungal; C, *Candida*; A, *Aspergillus*

The studies in Table II are not controlled trials, hence the recommendation of the Infectious Diseases Society of America that the routine use of granulocyte transfusions cannot be recommended (Hughes *et al*, 2002). Adequately powered prospective trials, with clinically relevant endpoints, precise patient selection criteria with generally accepted definitions of neutropenia are required before granulocyte transfusions can become part of routine clinical practice. However there are certain situations during neutropenia where the use of G-CSF-stimulated granulocyte transfusions may be reasonable. These include:

1 A resistant severe clinical infection in a neutropenic (neutrophil count $<0{\cdot}2$–$0{\cdot}5 \times 10^9$/l) patient that has shown no response to aggressive antibiotic treatment with no recovery in neutrophil count expected for more than 7 d.
2 Severe infections, e.g. systemic fungal infections/necrotizing fasciitis or severe neutropenic typhlitis progressing on appropriate anti-fungal or broad spectrum antibiotics, in neutropenic (neutrophil count $<0{\cdot}2$–$0{\cdot}5 \times 10^9$/l) patients, and no recovery in neutrophil count expected for more than 7 d.

Certain clinical situations are worthy of particular comment: neonatal sepsis and invasive fungal infections. Preterm neonates have immature granulopoiesis and a limited capacity for progenitor cell proliferation. This results in neutropenia during septic episodes. Transfusion of granulocytes may therefore help to reduce mortality and morbidity. Mohan and Brocklehurst (2003) used the Cochrane Database and other sources to identify randomized or comparative studies of granulocyte infusions with placebo, no granulocytes or intravenous immunoglobulin. They identified four trials. In three trials, 44 infants were randomized to granulocyte transfusions or placebo/no transfusion. No statistical difference in mortality was identified. In the fourth study, there was a marginal benefit for granulocyte infusions when compared with intravenous immunoglobulin. The side-effects associated with granulocyte infusions in the neonate were low. The authors concluded that there was inconclusive evidence to *support or refute* the routine use of granulocyte infusions in this setting. Granulocyte transfusions have been used by a number of groups for the treatment of systemic fungal infections (Grigg *et al*, 1996; Grigull *et al*, 2002; Singer *et al*, 2003) and as secondary prophylaxis (Kerr *et al*, 2003). However, as for other situations, small numbers of patients have been selected and this approach has not been subjected to the rigorous clinical evaluation that the newer anti-fungal agents have been subjected to (Herbrecht *et al*, 2002). Indeed, the trials with anti-fungal agents emphasize the need for the randomization of a large number of patients with predefined clinical endpoints to enable evaluation of these drugs. The cost and logistics of a similar trial with G-CSF-mobilized donor granulocytes may be prohibitive. The fact that current antifungal therapy is suboptimal and very expensive could be an impetus for a prospective randomized study. Granulocyte transfusions have been successfully used in rapidly progressing typhlitis (Hubel *et al*, 2002), and in patients with chronic granulomatous disease and liver abscesses (Fanconi *et al*, 1985; von Planta *et al*, 1997).

The suggested clinical indications above are largely derived from clinical experience and published data. Often granulocyte transfusions are commenced in the very ill and in situations where imminent bone marrow recovery is unlikely. However, there are important issues to consider, including problems with identifying suitable donors and, subsequently, of donor exhaustion. It is unrealistic to undertake granulocyte transfusions if the recipient is not expected to recover their marrow function within a reasonable time period. We suggest a 7-d cut-off point, as in our clinical experience we have been able to practically sustain transfusions over this time period without donor exhaustion.

An important consideration is whether patients who receive granulocyte infusions during induction/consolidation phases of chemotherapy and then subsequently proceed to transplantation have a delay in their engraftment. However, no published data have addressed this issue. It is unlikely that allosensitization to mature granulocytes will give rise to antibodies that cross-react with long-term progenitor cells responsible for stem cell engraftment. However, the administration of G-CSF to normal donors will invariably mobilize some progenitor cells into the peripheral blood and alloimmunization therefore remains a theoretical possibility. Further studies addressing this question are warranted.

Data published in abstract form alone suggest that prophylactic granulocyte infusions post-allogeneic BMT may reduce antibiotic utilization, febrile days and may improve survival (Adkins *et al*, 1999; Blum *et al*, 2001). However, larger randomized studies are required to fully evaluate the use of prophylactic G-CSF-mobilized donor granulocytes, with or without progenitor cells, in this scenario. In a recent study of murine haematopoietic stem cell transplantation (BitMansour *et al*, 2002), cotransplantation of lineage-restricted progenitors, known as common myeloid progenitors (CMP) and granulocyte-monocyte progenitors (GMP), protected against death following otherwise lethal challenge with *Aspergillus* and *Pseudomonas* species. Co-transplantation of CMP/GMP resulted in a significant and rapid increase in the absolute number of myeloid cells in the spleen, most of which were derived from the donor CMP/GMP. Furthermore, despite persistent peripheral neutropenia, improved survival correlated with the measurable appearance of progenitor-derived myeloid cells in the spleen. A marked reduction or elimination of tissue pathogen load was confirmed by culture and correlated with survival. These results suggest that enhanced reconstitution of a tissue myeloid pool offers protection against lethal challenge with serious fungal and bacterial pathogens. This study lends support to the further evaluation of prophylactic infusions of granulocytes together with myeloid progenitors to prevent serious neutropenia-associated infections. However, it should be noted that the murine model might not translate to the

human setting, as splenic haemopoiesis is absent in adult humans. Nonetheless, this approach may merit investigation in ABO, Rh D-matched individuals who are candidate for HLA-matched allogeneic transplantation. In such cases, large numbers of granulocytes for future prophylactic use could be harvested in tandem with stem cell collection.

## Adverse effects and donor selection

An important concern is the possibility of alloimmunization to HLA class 1 or granulocyte-specific antigens or both after transfusion. Although rapid alloimmunization does not seem to occur in patients who are severely immunosuppressed it may result in refractoriness to transfusion, both qualitatively and quantitatively (McCullough *et al*, 1986; Stroncek *et al*, 1996a) and severe non-haemolytic febrile transfusion reactions. Screening for leucoagglutinins prior to each transfusion is recommended, although reliable detection requires sophisticated tests that are not available in most centres. Common transfusion reactions include mild to moderate fever and chills with minor arterial oxygen desaturations, which may require slowing of the transfusion, anti-pyretics, anti-histamines and steroids (Price *et al*, 2000). More severe reactions, occurring in approximately 1–5% of transfusions include hypotension, pulmonary infiltrates and respiratory distress. Transfusion related acute lung injury is thought to be caused by neutrophil aggregation in the pulmonary microvasculature because of preformed donor HLA antibodies against recipient antigens, and requires vigorous respiratory and haemodynamic support, and usually high-dose steroids. There was thought to be an association between pulmonary infiltration and Amphotericin B (Wright *et al*, 1981; Berliner *et al*, 1985), however this has not been subsequently confirmed (Dutcher *et al*, 1989). It is still common practice to separate administration times if these drugs are being given concurrently. Adverse events do not appear to be related to the transfused cell dose (Karp *et al*, 1982). The presence of concomitant platelet refractoriness, post-transfusion pulmonary infiltrates and frequent febrile transfusion reactions should alert the clinician to the possibility of alloimmunization. If further courses of donor granulocytes are indicated, formal investigation should be undertaken.

Adverse effects in donors after G-CSF and dexamethasone appear to be universally mild, with transient bone ache, headache, myalgia, insomnia and fatigue commonly reported (Anderlini *et al*, 1996; Stroncek *et al*, 1996b). The question regarding the long-term safety of G-CSF in donors, particularly in terms of leukaemogenic potential, has been raised. There are very limited data from studies that have followed-up normal donors. Cavallaro *et al* (2000) undertook a 3–6-year follow-up of more than 100 donors and showed that blood counts at a median follow-up of 40 months were within the normal range. The use of G-CSF for mobilization of normal stem cell donors has been undertaken for more than 10 years and so far, there is no evidence of associated leukaemogenic potential. There has also been a recent report suggesting that corticosteroids may increase the risk of cataracts in granulocyte donors (Ghodsi & Strauss, 2001). This finding, if confirmed, suggests that the use of steroids in this setting should be used with caution. The red cell sedimenting agent, hetastarch acts as a plasma expander and can cause transient hypertension with flushing and headache. Apheresis may result in a mild hypovolaemia, which can be corrected with saline infusion, while citrate toxicity from the anti-coagulant occasionally causes peri-oral parasthesiae and pins and needles. Platelet and neutrophil counts fall after apheresis but return to normal within 7 d. Issues relating to donor exhaustion are discussed above.

The criteria for granulocyte donor selection should be broadly in line with those used for other routine blood donations. The lower age limit would normally be 18 years, although in extenuating clinical circumstances younger donors may be considered, with appropriate informed consent from the parent/guardian. The upper age limit may also be extended in similar circumstances, although is generally considered appropriate to use donors under the age of 65 years. The required apheresis procedure for granulocyte donation would present a potential clinical risk for cardiac or cerebrovascular events in donors with pre-existing inflammatory, autoimmune or vascular disease, and as such should be avoided in these subjects. The guidelines relating to the risk of viral exposure in blood donors should be adhered to. The recent UK Blood Transfusion Service Guidelines excluding donors with a prior history of blood transfusion after 1980 (because of the risk of new variant Creutzfeldt–Jakob disease) should also be considered. Most centres will aim to use CMV seronegative donors when the recipient is also CMV negative, as blood leucocytes are reservoirs for CMV. Vij *et al* (2003) have shown that in patients receiving two prophylactic granulocyte infusions poststem cell infusion within the first week postallogeneic BMT, the donor CMV status had no impact on CMV viraemia or disease. This study used donor granulocytes from sibling-matched BM doors and controls were patients without a sibling-compatible donor. They therefore suggest that with modern surveillance approaches to CMV infection, CMV-positive donors may be used if necessary. It should be pointed out that this study was undertaken in the context of prophylaxis and patients requiring infusions for established infections will require more than two infusions and therefore receive a higher viral burden. Furthermore, CMV infections are modulated by donor/ host alloreactivity and while CMV viraemia and infections may be low for the matched-sibling situation, this may not be true for multiple infusions from non-matched family and community members (Nichols *et al*, 2003). Until this study is confirmed in a wider setting, the use of CMV-negative donors should still be recommended.

Improvements in the technology of collection and the use of G-CSF now allow therapeutic doses of granulocytes to be routinely collected, however, efficient co-ordination and rapid response to requests for donation are important from family,

friends or the community. Future randomized controlled trials are needed and should be carefully planned to provide an answer to the question of efficacy of granulocyte transfusion and which subgroup of patients benefit the most. This may not be easy as large multinational studies are required. This will require common clinical protocols and common protocols for the procurement of granulocytes.

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