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Granulocyte transfusions: A concise review for practitioners

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Abstract

Granulocyte transfusions (GTXs) have been used to treat and prevent infections in neutropenic patients for more than 40 years, despite persistent controversy regarding their efficacy. This narrative review attempts to complement recent systematic reviews by the Cochrane Collaboration and provide both historical context and critical assessment of the most significant clinical studies published over the years. The data suggest that properly collected and promptly infused granulocytes are active against infections, both bacterial and fungal. The most important question that remains unanswered is in which patients the administration of granulocytes will be beneficial. The preponderance of evidence suggests that granulocyte transfusions may be efficacious in few select cases as a temporizing measure to control an infection that is expected (or proven) to be refractory to optimal antimicrobial treatment, and that could otherwise be controlled by marrow recovery, which is expected to happen. In this regard, they are best considered a "bridge" that grants enough time for the recipient to develop their own response to the infection. The challenges to use GTXs successfully are both clinical, in terms of timely identifying the patients who may benefit, and logistical, in terms of optimal selection of donors and collection technique.

Key Words: *granulocyte transfusion, invasive fungal infection, pulmonary reaction, neutropenia, granulocytopenia, gram-negative bacteremia*

Introduction

There are many more published reviews on GTXs than clinical trials: a simple PubMed search with the words "granulocyte transfusion" OR "granulocyte transfusions" in the "Title" field shows 47 Clinical Trials and 85 Reviews (search performed May 8, 2017). The aim of the current addition to the already overcrowded literature is to provide practicing clinicians with a succinct critical assessment of the data from the standpoint of an Infectious Disease practitioner who has worked for more than two decades in one of the institutions that pioneered this therapeutic modality.

Unbiased, systematic reviews have recently been published by the Cochrane Collaboration on the use of GTXs for prophylaxis and treatment. The conclusions were that there is low quality evidence suggesting GTX may work for prophylaxis [1] and that there is not enough evidence to decide on treatment efficacy [2]. These systematic reviews include, for methodological reasons, only 12 and 10 articles, respectively. In this review I will comment on most controlled trials on GTX, starting with the most recent ones, as well as on some case series that provide additional information. After discussing efficacy I will address toxicity. Finally, I will try to make recommendations for use and for research based on the evidence presented.

Brief history of GTXs

GTXs may be considered the oldest form of cell therapy. Injection of "buffy coat" preparations to treat neutropenic states was initially reported back in 1934 [3]. Subsequent studies showed that granulocytes infused into aplastic dogs migrated to sites of infection [4]. Animal models showed that GTXs could help in the management of bacterial infections [5]. However, obtaining enough neutrophils from healthy donors to produce a measurable increase in absolute neutrophil count (ANC) was challenging. This prompted using as donors patients with chronic myelogenous leukemia, who had ANC of up to 300,000/μL [6]. The subsequent development of the continuous blood-flow separator in 1969 provided a way to obtain enough granulocytes from healthy volunteers to establish granulocyte transfusion as a viable procedure (for a review

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of the history of the device see Freireich [7]). Case series and case reports suggesting a favorable effect in neutropenic patients with infection were published [8], and subsequently randomized controlled trials involving patients with (predominantly) bacterial infection during neutropenia were performed. Some of the studies showed improved outcomes [9–11], but others were negative [12,13] . Besides conflicting evidence regarding efficacy, data showing significant toxicity in the form of lethal pulmonary reactions also appeared [14]. The result was that by the 1990s the use of GTXs had decreased based on the widespread belief that GTXs did not add significant efficacy to optimal antimicrobial therapy and the practice became less common. For a critique of these early studies see Strauss [15].

Renewed interest in GTXs followed the availability of colony-stimulating factors (granulocyte colony-stimulating factor [G-CSF) was approved by the US Food and Drug Administration [FDA] in 1991) that, when administered to the donor, could result in much higher yields of granulocytes for transfusion [16]. If the reason for the negative results of some trials was insufficient dose, as some experts had postulated [15], the use of G-CSF stimulation should overcome the problem. The addition of dexamethasone to G-CSF was shown to increase the yield even more (by a factor of 1.5x) [17]. Since then, the approach in many US centers has been to use the combination of G-CSF and dexamethasone [18]. This approach is not universal, however, and several centers in Europe do not use G-CSF [19]. Overall, there seem to be significant technical differences from center to center nationally and internationally [18,19] and these could be a persistent source of differences in observed outcomes.

Technical considerations

There is general agreement that at least 1×10^{10} granulocytes (or 1.5×10^{8} granulocytes/kg) should be given per transfusion to expect efficacy, although there is only scant clinical evidence that this is the case. Many experts believe that even higher numbers are necessary or desirable —at least 4×10^{10}. The term "high-dose" granulocyte transfusion has been used to refer to $\geq 0.6 \times 10^{9}$ granulocytes/kg (which, in a 70 kg recipient, would give the 4×10^{10} mentioned above) [20]. The usual method to obtain granulocytes for transfusion in the US is by single-donor apheresis (intermittent or continuous centrifugation leukapheresis, using an agent like dextran or heptastarch to facilitate separation of the red blood cells). An adult therapeutic dose of granulocytes obtained by apheresis contains between 1.5×10^{8} and 3×10^{8} granulocytes/kg body weight of the designated recipient [21].

Besides apheresis, granulocytes may be obtained from the blood by centrifugation and collection of the "buffy coat" (the layer between the red blood cells and plasma) that results in a product rich in platelets and less abundant in granulocytes. A modification of this approach results in less contamination with red blood cells and plasma, and has been shown to be safe in a multicenter trial: The UK National Health System offers "Leucocytes, Buffy Coat, Irradiated". Each pack is approximately 50 mL, has a hematocrit of 45% and contains $1–2 \times 10^{9}$ white blood cells, 90×10^{9} platelets and 9.5 g of hemoglobin [22].

Finally, filtration leukapheresis was used in several of the original studies of GTX because it allowed the collection of large quantities of granulocytes [9–11]. The cells showed impaired phagocytic activity *in vitro* [23] and transfusion of granulocytes obtained by filtration apheresis was associated with more side effects, so the procedure seems to have been abandoned.

Studies on the activity of granulocytes collected for transfusion suggest the cells generally remain functional for a few hours [23–25], although many timed variations in gene expression may be found depending on the collection method (stimulation of donors with dexamethasone or G-CSF or both) and storage [26], and some abnormalities in function (e.g., impaired killing of Candida yeast forms) can be detected *in vitro* [27]. Ideally, transfusion should take place less than 6 h after collection. It is customary to irradiate the cells before transfusion to prevent transfusion-associated graft-versus-host disease (TA-GVHD), which could potentially be caused by lymphocytes in the collected product. Some experts, however, believe this compromises neutrophil function and unirradiated granulocytes can be used safely. A controlled trial of irradiated versus nonirradiated GTX did not find any difference and there were no cases of TA-GVHD [28].

GTX in current clinical practice: case report

Figure 1 illustrates the effect of GTXs on a proven invasive fungal infection that seemed to be progressing on antifungal therapy. A 9-year-old child with severe aplastic anemia presented with fever, positive blood cultures for *Fusarium solani* and a wedge-shaped, dense pulmonary infiltrate that showed branching septate hyphae on a fine-needle aspirate. The apparent lack of response of the infection to the combination of voriconazole and amphotericin B and the subsequent resolution after a few GTXs (with appearance of the crescent sign, which is associated with neutrophil recovery) are evident from the images (this patient was included in the series of *Fusarium* infections reported by National Institutes of Health