Granulocyte transfusions in severe aplastic anemia

Roma V. Rajput,[1] Vaani Shah,[2] Ruba N. Shalhoub,[3] Kamille West-Mitchell,[4] Nu Ri Cha,[4] Cathy Conry-Cantilena,[4] Susan F. Leitman,[4] David J. Young,[5] Brian Wells,[6] Georg Aue,[6] Cynthia E. Dunbar,[5] Bhavisha A. Patel,[1] Richard W. Childs,[1] Neal S. Young,[1] Colin O. Wu,[3] Emma M. Groarke[1#] and Shelley S. Kalsi[2#]

[1]Hematology Branch, National Heart, Lung, and Blood Institute, NHLBI; [2]Hematology Consult and Graduate Medical Section, NHLBI; [3]Office of Biostatistics Research, NHLBI; [4]Department of Transfusion Medicine, Clinical Center; [5]Translational Stem Cell Biology Branch, NHLBI and [6]Laboratory of Transplantation Immunotherapy, NHLBI, National Institutes of Health, Bethesda, MD, USA

[#]EMG and SSK contributed equally as senior authors.

Correspondence: R.V. Rajput
roma.rajput@nih.gov

Received: June 28, 2023.
Accepted: November 30, 2023.
Early view: December 7, 2023.

https://doi.org/10.3324/haematol.2023.283826

Abstract

Patients with severe aplastic anemia (SAA) are at high risk of morbidity and mortality due to severe infections. We aimed to characterize the role of granulocyte transfusions (GT) in SAA. Primary outcomes were survival after the first GT, including overall survival (OS) at last follow up, survival to discharge, and receipt of a hematopoietic stem cell transplant (HSCT) Secondary outcomes included evaluation of clinical response at 7 and 30 days after initiation of GT, using a clinical scoring system incorporating microbiological and radiographic response. Twenty-eight SAA patients underwent 30 GT courses with a per-dose median of $1.28x10^9$ granulocytes/kilogram (range, 0.45-$4.52x10^9$). OS from initial GT to median last follow up (551 days) was 50%, with 39% (11/28) alive at last follow up. Sixty-four percent (18/28) of all patients survived to hospital discharge. Patients with a complete or partial response, or stable infection, at 30 days had significantly better OS compared to non-responders (P=0.0004). Eighty-six percent (18/21) of patients awaiting HSCT during GT underwent a transplant and 62% (13/21) survived to post-HSCT discharge. Sex, type of infection, and percentage of days with absolute neutrophil count >$0.2x10^9$/L during the course of GT were not predictive of survival (P=0.52, P=0.7 and P=0.28, respectively). Nine of 28 (32%) patients developed new or increased human leukocyte antigen alloimmunization during their GT course. GT in SAA may have an impact on survival in those patients with improvement or stabilization of their underlying infection. Alloimmunization can occur and OS in this population remains poor, but GT may be a useful tool to bridge patients to curative treatment with HSCT.

Introduction

Patients with severe aplastic anemia (SAA), a life-threatening immune-mediated bone marrow failure disorder characterized by destruction of hematopoietic stem cells, are at high risk of severe infection due to prolonged neutropenia. Hematopoietic recovery can be difficult to achieve and severe infections lead to significant morbidity and mortality. Reports, including a 10-year review of SAA patients at our institution who received granulocyte transfusions (GT), have shown a link between clinical response after initiating GT and survival, suggesting an adjunctive role for GT in managing infections in this population.[1,2] Results from a prospective, multicenter, randomized controlled trial seeking to determine the efficacy of GT in patients with severe neutropenia demonstrated no significant difference in survival and microbial response between patients who received GT with standard antimicrobial therapy and those who received standard antimicrobial therapy alone.[3] The limitations of that trial included low patient accrual, leaving the study underpowered to detect a true effect of GT, and below-target GT doses, resulting in differences in success rates between those who received higher *versus* lower GT doses. Furthermore, only a few subjects had SAA, all of whom were assigned to the control treatment arm, limiting our interpretation of the data in the SAA population.

We aimed to provide an updated and expanded report of our institution's experience with GT, characterizing the clinical outcomes of patients with SAA who received GT, focusing on clinical response, patients' survival, and ability to bridge patients to curative treatment with hematopoietic stem cell transplant (HSCT).

Methods

Patients

All patients with SAA who received a minimum of one GT from 2010-2020 were included in this study. SAA patients all met modified Camitta criteria, that is, bone marrow cellularity of <30% and at least two of the following blood counts: an absolute neutrophil count (ANC) of 0.5×10^9/L or less, an absolute reticulocyte count of 60×10^9/L or less, and a platelet count of 20×10^9/L or less. Patients were enrolled on the following clinical trials at the time of GT: NCT00604201, NCT01174108, NCT01193283, NCT01623167, NCT03173937, and NCT03520647. The above-mentioned trials were approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute. All patients or guardians provided written informed consent to participation in the trials.

Clinical data were collected from electronic medical records or from clinical trial databases. Baseline demographic information included age, sex, initial SAA therapy, disease severity, number of rounds of immunosuppressive therapy (IST) received, ABO type, and presence of human leukocyte antigen (HLA) class I and class II antibodies prior to and after GT courses. All patients had significant infections prompting initiation of GT and type of infection, pathogen isolated, and site of infection were recorded.

Granulocyte product data included number of GT, the patient's weight at initial GT, granulocyte dose of each product in cells, donor ABO type, donor HLA type, and donor testing for agents of transfusion-transmissible infections. ANC prior to GT, number of days that the ANC was greater than $0.2x10^9$/L, and percentage of days with available ANC data were assessed. If multiple ANC were collected daily, the first available post-transfusion ANC drawn the morning after transfusion was used for analysis.

Patients were eligible to receive a GT if they had the following: (i) proven or probable invasive fungal disease determined by the clinical team, or (ii) a bacterial infection which, in the experience of our center, was associated with greater than 90% mortality, as well as (iii) an ANC of less than 0.2×10/L, and (iv) no response to appropriate antibiotic or antifungal therapy for 24-48 hours. The duration of a GT course was determined by multiple factors including neutrophil response to infection, clinical response or progression of infection, development of transfusion reactions, HLA alloimmunization, and the availability of granulocyte donor products for same-day administration.

Donor selection, granulocytapheresis, and transfusion

Eligible granulocyte donors were enrolled on the NCT01553214 trial. After giving informed consent, donors received a single 480 μg subcutaneous injection of filgrastim 12-24 hours prior to leukapheresis and 8 mg of oral dexamethasone 12 hours prior to leukapheresis. Granulocyte concentrates were collected using a blood separator (CS3000 Plus, Fenwal, Deerfield, IL, USA) processing 7 L of whole blood with trisodium citrate anticoagulant (Citra Anticoagulants, Braintree, MA, USA) and 6% hetastarch (He span, Braun Medical, Irvine, CA, USA). Granulocyte concentrates were sedimented by gravity following collection to reduce red cell content if there was major ABO incompatibility. All granulocytes were irradiated and transfused within 10 hours of collection.

Outcome measures

Primary outcomes were overall survival (OS) following the first GT to last follow up, survival to hospital discharge following hospitalization for a course of GT, and percentage of patients who received an infusion of hematopoietic stem cells among those who were awaiting HSCT during the GT course. Secondary outcomes included clinical responses at 7 and 30 days after initiation of GT and association between the following variables and OS: sex, infection type, duration of ANC over 0.2×10^9/L during the GT course, presence of HLA alloimmunization, and granulocyte product characteristics (granulocyte dose, ABO matching).

A GT course was defined as the number of days from first transfusion to 3 days after the last transfusion to account for the effect of the last transfusion. Additional outcomes included rate of HLA alloimmunization after GT, rate of successful HSCT in patients with transplant-donor specific antibodies after GT, and rate of GT complications including GT reactions and transfusion-transmitted virus positivity in previously negative granulocyte donors.

Response was characterized using a scoring system based on response (determined by the physician and including defervescence, hemodynamic stability, and improvement in infection-related symptoms), radiographic response (decrease in size of infection on imaging), and microbiological response (resolution of bacteremia, if applicable). Complete response and partial response were defined as improvements in all three and one or two criteria, respectively. Stable disease was defined as no improvement and progressive disease was identified by evidence of a breakthrough infection or clinical deterioration. GT reactions were identified by severity and imputability based on the National Healthcare Safety Network Biovigilance Component Hemovigilance Module Protocol.[4]

Statistical analysis

Summary statistics are described as the median with range for continuous variables, and frequency with proportion for categorical variables. Kaplan-Meier estimators were used to compare OS distributions based on the type of 30-day response to GT. Cox proportional hazard models were used to analyze the effect of covariates on survival time. Five courses were omitted from the ANC analysis because of excessive missingness of recorded data. OS was determined from the time of the first GT. Surviving patients were censored at their last follow-up date. *P*

values for covariate effects were calculated using the log-rank test. All statistical analyses were performed using R (version 4.0.2).

Results

Twenty-eight SAA patients with a median age of 20 years (range, 6-65 years) underwent 30 GT courses. Initial IST included horse antithymocyte globulin/cyclosporine and eltrombopag (n=11), horse antithymocyte globulin/cyclosporine (n=8), cyclophosphamide/cyclosporine (n=6), and horse antithymocyte globulin/cyclosporine plus sirolimus (n=1). Two patients did not receive IST and went directly to HSCT. A median of eight granulocyte products per course (range, 1-39 products) were administered over a median of 23.5 days (range, 3-103 days), with a per-dose median of $1.28x10^9$ cells/kg (range, 0.45-$4.52x10^9$ cells/kg). Indications for GT included invasive bacterial (n=14), fungal (n=13) and mixed (n=3) infections. The patients' characteristics are summarized in Table 1. Types of infections, organisms, and sites of infection are summarized in *Online Supplementary Table S1*.

Response

Overall, 86% (25/29) of infections remained stable or responded to GT. Over a GT course, complete responses were observed in 23% (7/30) on day 7 after the initial GT, and increased to 45% (13/29) on day 30. Most of the patients with fungal infection had a partial response (6/13; 46%) or stable infection (5/13; 38%) at 7 days, with only one complete responder; these responses improved to complete (7/13; 54%), and partial (5/13; 38%) by day 30. Only one patient with a fungal infection had progressive disease, which was evident early by day 7. Patients with bacterial infections had similar numbers of responses at days 7 and 30, although three patients had worsening bacterial infection by day 30 (Table 2).

Survival

After completion of their GT course, 64% (18/28) of patients survived to hospital discharge. The rate of survival to discharge in GT recipients who achieved complete response by day 30 was 85% (11/13) (Table 2). The OS rate from initial GT was 50% at a median duration of follow up of 551 days (range, 2-4,213 days) (Figure 1A). Overall, 39% (11/28) of patients were alive at last follow up. Patients who achieved a complete or partial response, or had a stable infection at 30 days had significantly better OS than that of non-responders (P=0.0004) (Figure 1B).

Predictors

Sex, weight, type of infection, percentage of days on which the ANC was greater than $0.2×10^9$/L, ABO donor-recipient mismatch, mean granulocyte dose, and HLA alloimmunization (negative HLA antibodies at baseline to positive HLA antibodies after GT or increased panel-reactive antibodies in those alloimmunized at baseline) were not predictive

Table 1. Demographics of the patients and characteristics of the granulocyte products.

Parameter	Result
Patients, N (%)	28 (100.0)
Sex, N (%)	
Male	15 (53.6)
Female	13 (46.4)
Age in years, N (%)	
<18	13 (46.4)
18-39	7 (25.0)
40-59	6 (21.4)
>60	2 (7.2)
Weight, kilograms, median (range)	59 (15-118)
Received IST,* N (%)	26 (92.9)
ATG + CSA + EPAG	11 (42.3)
ATG + CSA**	9 (34.6)
Cyclophosphamide + CSA	6 (23.1)
Required >1 round of IST	8 (30.8)
Received HSCT, N (%)	23 (82.1)
Haploidentical-cord	10 (43.5)
Matched unrelated donor***	4 (17.4)
Matched related donor	6 (26.1)
Expanded cord	1 (4.3)
Unknown	2 (8.7)
HSCT donor-recipient CMV match, N (%)	
Matched	20 (83.3)
Mismatched	1 (4.2)
Unknown	3 (12.5)
HSCT donor-recipient ABO compatibility, N (%)	
Matched	12 (50.0)
Major mismatched	5 (20.8)
Minor mismatched	3 (12.5)
Bidirectional	2 (7.2)
Unknown	2 (7.2)
Baseline ANC prior to GT, N	
0.0-0.09$x10^9$/L	26
0.1-0.2$x10^9$/L	2
Percentage of days with ANC >0.2$x10^9$/L (median, range)	50 (11-95)
Total N of granulocyte courses	30
Granulocyte products per course, days (median, range)	8 (1-39)
Granulocyte dose, cells/kg, median (range)	1.3$x10^9$ (0.45-4.52$x10^9$)
Time from first day of documented infection to GT in days, median (range)	5 (0-20)

*Two patients did not receive immunosuppressive therapy and went directly to hematopoietic stem cell transplant. **One patient received sirolimus in addition to antithymocyte globulin and cyclosporine. ***One of the two patients who received two granulocyte courses received transplants from two matched unrelated donors. N: number; IST: immunosuppressive therapy. ATG: antithymocyte globulin; CSA: cyclosporine; EPAG: eltrombopag; HSCT: hematopoietic stem cell transplant; CMV: Cytomegalovirus; ANC: absolute neutrophil count; GT: granulocyte transfusions.

Table 2. Responses at days 7 and 30 after initiation of granulocyte infusion *versus* survival to hospital discharge: overall and by type of infection.

	Day 7 response N (%)	Survival to hospital discharge, N/N (%)	Day 30 response N (%)	Survival to hospital discharge, N/N (%)
	All infections, N=30 (day 7) or 29 (day 30)*			
Type of response				
Complete	7 (23)	4/7 (57)	13 (45)	11/13 (85)
Partial	12 (40)	8/12 (67)	11 (38)	7/11 (64)
Stable	9 (30)	7/9 (78)	1 (3)	1/1 (100)
Progressive	2 (7)	0/2 (0)	4 (14)	0/4 (0)
Overall	-	-	-	18/28* (64)
	Bacterial, N=14			
Type of response				
Complete	5 (36)	3/5 (60)	6 (43)	5/6 (83)
Partial	6 (43)	4/6 (67)	4 (29)	3/4 (75)
Stable	3 (21)	2/3 (67)	1 (7)	1/1 (100)
Progressive	0 (0)	0/0	3 (21)	0/3 (0)
Overall	-	-	-	10/15 (67)
	Fungal, N=13			
Type of response				
Complete	1 (8)	0/1 (0)	7 (54)	6/7 (86)
Partial	6 (46)	4/6 (67)	5 (38)	2/5 (40)
Stable	5 (38)	4/5 (80)	0 (0)	0/0
Progressive	1 (8)	0/1 (0)	1 (8)	0/1 (0)
Overall	-	-	-	8/13 (62)
	Mixed, N=3*			
Type of response				
Complete	1 (33)	1/1 (100)	0 (0)	0/0
Partial	0 (0)	0/0	2 (100)	2/2 (100)
Stable	1 (33)	1/1 (100)	0 (0)	0/0
Progressive	1 (33)	0/1 (0)	0 (0)	0/0
Overall	-	-	-	2/3 (67)

N: total granulocyte courses administered in 28 patients. Response categorized as complete: improvement in all three criteria (clinical, radiographic, and microbiological); partial: improvement in one or two criteria; stable: no improvement; and progressive: clinical decline or breakthrough infection. *One patient who died before the 30-day response assessment had mixed bacterial/fungal infection.

of survival (Table 3, *Online Supplementary Table S2*, *Online Supplementary Figure S1*).

Hematopoietic stem cell transplantation outcomes

Of 21 patients who were awaiting HSCT during their GT course, 18 (86%) successfully started conditioning. Three patients did not receive HSCT; two patients were ineligible because of their critical clinical status and ultimately died and one patient lacked a suitable donor but achieved hematopoietic recovery after IST and survived. Thirteen of the 21 (62%) awaiting HSCT survived to discharge from hospital. Of the eight patients awaiting HSCT who did not survive to hospital discharge, six received HSCT and died due to progression of the initial infection (n=5) or development of a new infection (n=1). Infections that led to death included disseminated Klebsiella infection (n=2), adenovirus (n=2), trichosporonosis (n=1), and aspergillosis (n=1). Twelve of 16 patients (75%) who were awaiting HSCT at the time of their GT course with available engraftment data successfully engrafted (*Online Supplementary Table S5*).

Five out of 28 patients (18%) were HLA-alloimmunized at baseline. All five (100%) demonstrated an increase in panel-reactive antibodies after GT. Of the 23 (82%) patients not alloimmunized at baseline, four (17%) developed HLA antibodies during or after GT. In total, nine of 28 (32%) patients developed new or increased HLA alloimmunization (*Online Supplementary Table S3*); four developed donor-specific antibodies to potential HSCT donors (*Online Supplementary Table S4*), three did not develop donor-specific antibodies, and two did not have available HSCT donor HLA data. All four patients who developed donor-specific antibodies to their originally planned donor successfully underwent HSCT using an alternative donor without development of graft failure; two of these patients ultimately died from progression of their initial infection. Three patients had primary or secondary graft failure; none of these patients

had HLA alloimmunization at baseline or that developed alloimmunization during the GT course (*Online Supplementary Table S5*).

Safety

Nineteen of the 28 (68%) patients met criteria for non-severe febrile non-hemolytic transfusion reaction during their GT course. Two patients developed an allergic reaction which was treated with antihistamines with resolution of symptoms. One patient met criteria for severe, transfusion-associated circulatory overload of probable imputability based on National Healthcare Safety Network guidelines. The patient had underlying cardiomyopathy and died 3 months after his last GT due to systemic adenovirus infection in the setting of primary graft failure after HSCT. No donor tested positive for agents of transfusion-transmissible infection.

Discussion

This study is a follow up to a previous report from our institution detailing the largest clinical experience utilizing GT in SAA patients with invasive fungal and/or bacterial infections, and showed a 64% rate of survival to hospital discharge, similar to the 58% reported in the previously studied cohort, with a significantly higher OS rate in patients with a stable infection or improved response compared to those with progression of infection.[1] We aimed to estimate the OS based on clinical response to GT at 30 days and found a significantly higher OS in patients with a stable infection or improved response compared to those with progression of infection.

To our knowledge, this is the first analysis of long-term survival and HSCT-related outcomes in SAA patients receiving GT. Given the progressive and refractory nature of these infections prior to the initiation of GT, and that uncontrolled infections are contraindications to HSCT, we believe that the high rates of receipt of HSCT in those awaiting HSCT (86%, 18/21) and survival to hospital discharge after HSCT (75%, 13/18) are largely attributable to the effect of GT. Although some patients developed HLA antibodies, even some to their originally planned donors, all were able to undergo HSCT.

Our analysis did not reveal an association between type of infection and OS, there being similar rates of survival to hospital discharge in patients with invasive fungal or bacterial infections. In our previous cohort, 44% of patients with invasive fungal or bacterial infections survived to hospital discharge compared to 62% in this current study. It has been shown that over time infection-related mortality has decreased significantly in SAA patients unresponsive to IST, likely due to improvements in supportive care and use of alternative donor transplants.[5] A study evaluating the efficacy and safety of posaconazole as primary prevention of invasive fungal disease in patients with SAA demonstrated a significantly lower incidence of such fungal diseases in patients treated with posaconazole compared to the incidence in a control group.[6] In contrast to the prior standard of care, the emergence and prophylactic use of this broad-spectrum oral antifungal agent may be a contributing factor to the observed increase in SAA patients who survived to hospital discharge.

To date, there has been inconclusive evidence of the efficacy of GT in SAA and other at-risk populations due to several limitations including variable granulocyte collection methods, poor patient accrual in randomized controlled trials, and physician bias with randomizing patients to GT due to pre-conceived beliefs about the efficacy of these transfusions.[1,3,7] A Cochrane review of ten randomized controlled trials with 587 patients evaluating efficacy of GT *versus* standard antimicrobial therapy alone found insufficient evidence that GT affected all-cause mortality.[8] The majority of the studies included in this review did not use granulocyte colony-stimulating factor (G-CSF) for donor stimulation, whereas this is now commonly given to granulocyte donors, and thus the transfused products had substantially lower granulocyte content. The RING trial,

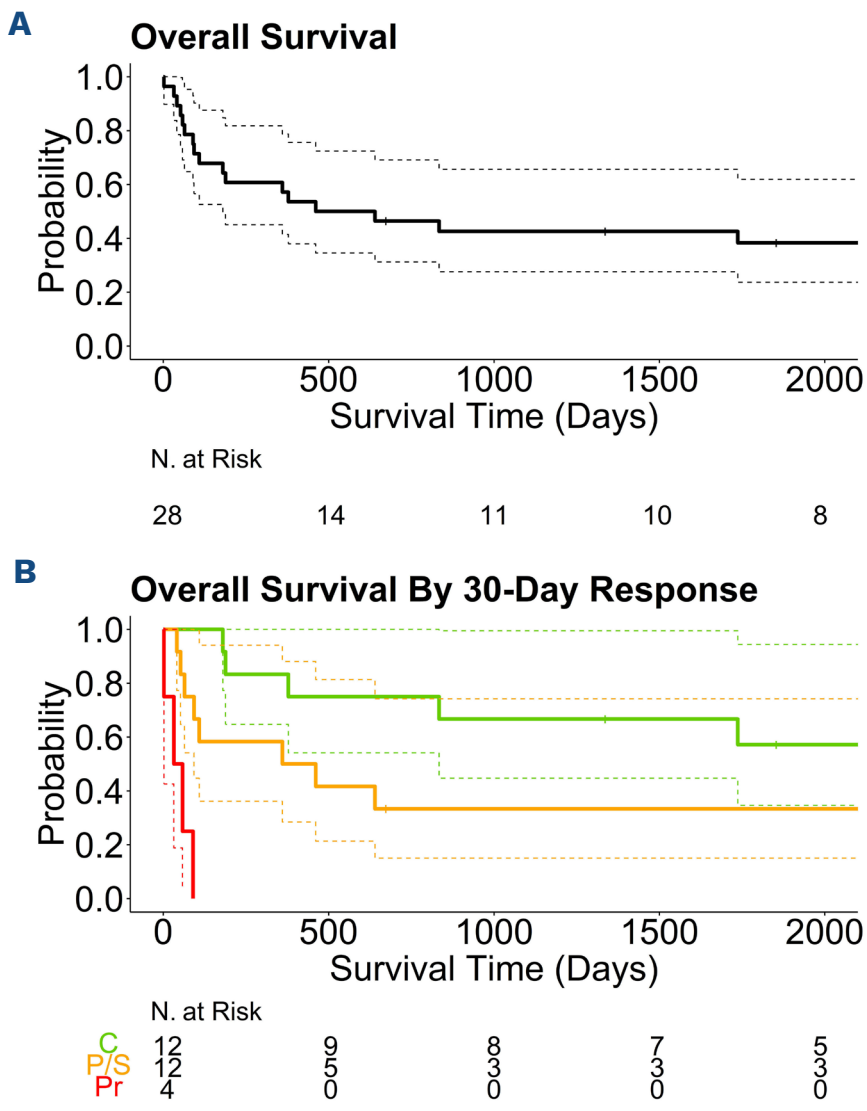

Figure 1. Overall survival of patients with severe aplastic anemia who received granulocyte transfusions. (A) Overall survival from the initial granulocyte transfusion. (B) Overall survival from the initial granulocyte transfusion based on overall response at 30 days after the granulocyte transfusions. C: complete response; P/S: partial response or stable infection; Pr: progression.

Table 3. Cox proportional-hazard models for time to death from first granulocyte transfusion (N=25).*

	Coefficient	Standard error	Hazard ratio	95% Confidence interval	*P*
Sex					
Male	0.35	0.55	1.42	0.48-4.20	0.52
Female	-	-	-	-	-
Weight	-0.004	0.009	1.00	0.98-1.01	0.62
Type of infection					
Bacterial	-	-	-	-	-
Fungal	0.42	0.54	1.52	0.53-4.37	0.44
Mixed	0.50	1.08	1.65	0.20-13.66	0.64
Percentage of days ANC >0.2x10^9/L*	-1.05	0.97	0.35	0.05-2.37	0.28
ABO % mismatch	-0.008	0.009	0.99	0.97-1.01	0.39
Mean granulocyte dose (cells/kg)	0.114	0.209	1.12	0.74-1.69	0.58
HLA-alloimmunization status pre-GT *vs.* post-GT					
Negative-Positive	0.085	0.775	1.09	0.24-4.97	0.91
Positive-Positive	0.169	0.627	1.18	0.35-4.05	0.79

*Five courses omitted from the analysis of absolute neutrophil counts because of excessive missingness of recorded counts. ANC: absolute neutrophil count; HLA: human leukocyte antigen; GT: granulocyte transfusions.

the most recent and largest randomized controlled trial to date, studied 114 patients with severe neutropenia and suspected or confirmed invasive infection who were randomized to receive granulocytes from donors stimulated with G-CSF and dexamethasone or standard antimicrobial therapy alone. This trial was limited by lack of power due to poor patient accrual, and below-target granulocyte dose in a third of patients.[3]

Although an established target granulocyte dose to achieve efficacy has not been identified, a Cochrane meta-analysis derived a minimum dose of 0.1x10^9 cells/kg as contributing to better clinical outcomes.[9] European guidelines similarly recommend a standard granulocyte apheresis product for adults to be 0.1-0.3x10^9 cells/kg of recipient body weight.[10] Siedel *et al.* reviewed 59 pediatric and young adults with severe neutropenia receiving a course of GT, including several patients with SAA, and concluded that a critical minimum GT dose of 0.3x10^9 cells/kg was needed to support the control of infections in high-risk neutropenic patients.[11] In contrast to the RING trial in which higher OS was observed in patients who received higher doses (>0.6x10^9 cells/kg) compared to lower doses, we did not observe an association between the mean granulocyte dose received per patient and survival.[3] Again in contrast to the RING trial, only 11% (3/28) of the patients in our cohort had mean granulocyte doses less than 0.6x10^9 cells/kg. Furthermore, the median granulocyte dose/kg received by our patients was multifold higher than all recommended dose targets, likely crossing the threshold beyond which there is no or limited association between GT dose and clinical outcome. While Siedel *et al.* utilized either prednisolone or G-CSF for donor stimulation to increase granulocyte product content, the use of both G-CSF and dexamethasone produces higher granulocyte product doses without increasing the incidence of donor side effects compared to those in donors receiving G-CSF or dexamethasone alone.[12,13] Furthermore, an association between G-CSF for donor stimulation and a theoretical risk of malignancy has been refuted even for donors undergoing multiple stimulations.[14] Based on these considerations concerning both donors and recipients, granulocyte collection centers should strive to achieve minimum granulocyte doses of 0.6x10^9/kg of recipient weight. Granulocyte apheresis product yields are maximized by using dual donor stimulation (dexamethasone plus G-CSF), and this dual stimulation regimen should be the standard to optimize the therapeutic effect of GT in patients with SAA.

One-third of our patients developed new or progressive HLA-alloimmunization during their GT course. Generally, HLA antibody testing in GT recipients is performed prior to the first transfusion, during the transfusion course if an adverse reaction occurs, and after the last transfusion.[15] Our study did not reveal an association between development of HLA-alloimmunization and OS in our granulocyte-treated SAA patients, similarly to what was seen in the RING trial cohort.[16] Published data have revealed the negative impact of donor-specific antibodies on engraftment of donor cells in patients who received transplants from unrelated or haploidentical donors.[17,18] Ideally, granulocyte donors who share HLA alleles with potential HSCT donors should be avoided, to prevent the development of donor-specific antibodies in the transfusion recipient. However, implementation of this strategy may not always be possible because of the urgent nature of the need for GT and the limited number of granulocyte donors. Fortunately, in our cohort, all four patients who developed donor-specific

antibodies against their original hematopoietic cell donor ultimately underwent HSCT after an alternative donor was identified. Physicians will need to assess the benefit of GT *versus* the risk of developing donor-specific antibodies on a patient-by-patient basis.

Transfusion reactions following GT have been widely documented; febrile and allergic reactions have been reported to occur at a rate of 10-80%.[3,19,20] While the rate of febrile non-hemolytic reactions in our cohort was within this reported rate range, all cases resolved after administration of antipyretics. Concomitant fever due to underlying infection may have led to an overestimate of true reactions to GT. Alternatively, the high rate of febrile non-hemolytic transfusions reactions may be related to the high dose of granulocytes in the transfused products. The rate of severe transfusion reactions in our cohort was similar to that in the most recent randomized controlled trial.[3] Overall, GT in SAA recipients appears to be safe with low rates of severe reactions.

As a retrospective, observational study, limited to selected SAA patients from a single institution, our findings may not be generalizable. Among SAA patients, those who were highly alloimmunized did not receive GT due to difficulty in identifying sufficient granulocyte donors who lacked the cognate antigens. Our conclusions, therefore, only apply to SAA patients who are not highly alloimmunized prior to GT. The recently published experience with GT in patients with chronic granulomatous disease at our institution described a similar response rate to GT but, in contrast to our SAA cohort, GT given prior to HSCT were associated with high rates of alloimmunization and primary graft failure.[21] A successful GT strategy requires a sophisticated transfusion medicine department, including a blood collection center with access to a large pool of highly dedicated and motivated granulocyte donors, and rapid turnaround of HLA testing, which can be both cost-prohibitive and labor intensive. As our facility is research-focused and government-funded, there were adequate resources to collect, process, and administer HLA-typed granulocyte products. Our procedures for collecting and transfusing granulocyte components may not be feasible at other institutions, limiting the generalizability of our findings and application of our approach. Although GT may not be readily available at every institution, our findings can lead to centers without the capability to administer GT to consider transfer of selected patients to a specialized center, and may even lead to consideration of investment in infrastructure to support administration of GT. In the absence of randomized controlled trials in SAA and using this particular technical approach to GT, our observational study provides valuable guidance for clinical practice. Statistical limitations included the low number of patients, which did not allow for multivariate analysis and may have affected the ability to detect differences in the univariate analyses of variables related to GT that affect OS.

Highlights of this updated review of SAA patients undergoing GT reveal the following. (i) GT can be offered to critically ill SAA patients with invasive infections. (ii) Adjunctive therapy with GT with continued advances in antimicrobial and antifungal therapy and other supportive care measures may help to increase OS. (iii) GT may be a particularly valuable tool in patients with life-threatening infections as a bridge to curative therapy with HSCT. (iv) HLA-alloimmunization and, specifically, donor-specific antibodies can occur and must be considered when making the decision to use GT. Avoidance of granulocyte products from donors who share HLA alleles with potential HSCT donors may mitigate this risk. (v) Transfusion reactions have been observed in SAA patients receiving GT but, in our cohort, these were generally mild and did not affect mortality.

Disclosures

NSY and CED have a cooperative research and development agreement with Novartis. The remaining authors declare that they have no competing financial interests.

Contributions

RVR conceptualized the study and wrote the manuscript. VS assisted in gathering data. RS analyzed results and created figures. EMG, SSK, NSY, and COW conceptualized and performed research. KW, NC, CC, SFL, DJY, CED, BAP, BW, GA, and RWC provided clinical care and assisted in collecting data. All authors edited and approved the final manuscript.

Funding

This research was supported by the Intramural Research Program of the National Institutes of Health, National Heart, Lung, and Blood Institute.

Data-sharing statement

De-identified data will be shared with other researchers upon reasonable request to the corresponding author. The sharing will require a detailed proposal to the study investigators and a data transfer agreement must be signed.

References

1. Quillen K, Wong E, Scheinberg P, et al. Granulocyte transfusions in severe aplastic anemia: an eleven-year experience. Haematologica. 2009;94(12):1661-1668.
2. Wang H, Wu, Y, Fu R, et al. Granulocyte transfusion combined with granulocyte colony stimulating factor in severe infection patients with severe aplastic anemia: a single center experience from China. PLoS One. 2014;9(2):e88148.
3. Price TH, Boeckh M, Harrison RW, et al. Efficacy of transfusion

with granulocytes from G-CSF/dexamethasone-treated donors in neutropenic patients with infection. Blood. 2015;126(18):2153-2161.
4. Center for Disease Control and Prevention. The National Healthcare Safety Network (NHSN) Biovigilance Component Hemovigilance Module Surveillance Protocol. https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf. Accessed November 20, 2023.
5. Valdez JM, Scheinberg P, Nunez O, et al. Decreased infection-related mortality and improved survival in severe aplastic anemia in the past two decades. Clin Infect Dis. 2011;52(6):726-735.
6. Chen M, Zhuang JL, Duan MH, et al. Posaconazole as primary prevention of fungal infection in intensive immunosuppressive therapy for severe aplastic anemia. Chin J Hematol. 2018;39(2):128-131.
7. Pammi M, Brocklehurst P. Granulocyte transfusions for neonates with confirmed or suspected sepsis and neutropenia. Cochrane Database Syst Rev. 2011;2011(10):CD003956.
8. Estcourt LJ, Stanworth SJ, Hopewell S, Doree C, Trivella M, Massey E. Granulocyte transfusions for treating infections in people with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2016;4(4):CD005339.
9. Stanworth SJ, Massey E, Hyde C, et al. Granulocyte transfusions for treating infections in patients with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2005;20(3):CD005339.
10. Guide to the preparation, use, and quality assurance of blood components. European Committee (Partial Agreement) on Blood Transfusion. 21st ed. European Directorate for the Quality of Medicines and Healthcare. 2023.
11. Seidel MG, Minkov M, Witt V, et al. Granulocyte transfusions in children and young adults: does the dose matter? J Pediatr Hematol Oncol. 2009;31(3):166-172.
12. Stroncek DF, Yau YY, Oblitas J, Leitman SF. Administration of G-CSF plus dexamethasone produces greater granulocyte concentrate yields while causing no more donor toxicity than G-CSF alone. Transfusion. 2001;41(8):1037-1044.
13. Liles WC, Huang JE, Llewellyn C, SenGupta D, Price TH, Dale DC. A comparative trial of granulocyte-colony-stimulating factor and dexamethasone, separately and in combination, for the mobilization of neutrophils in the peripheral blood of normal volunteers. Transfusion. 1997;37(2):182-187.
14. Shaw BE, Confer DL, Hwang W, Pulsipher MA. A review of the genetic and long-term effects of G-CSF injections in healthy donors: a reassuring lack of evidence for the development of haematological malignancies. Bone Marrow Transplant. 2015;50(3):334-340.
15. Cugno C, Deola S, Filippini P, Stroncek DF, Rutella S. Granulocyte transfusions in children and adults with hematological malignancies: benefits and controversies. J Transl Med. 2015;13(1):362.
16. Price TH, McCullough J, Strauss RG, et al. WBC alloimmunization: effects on the laboratory and clinical endpoints of therapeutic granulocyte transfusions. Transfusion. 2018;58(5):1280-1288.
17. Spellman S, Bray R, Rosen-Bronson S, et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. Blood. 2010;115(13):2704-2708.
18. Ciurea SO, de Lima M, Cano P, et al. High risk of graft failure in patients with anti-HLA antibodies undergoing haploidentical stem-cell transplantation. Transplantation. 2009;88(8):1019-1024.
19. West KA, Conry-Cantilena C. Granulocyte transfusions: current science and perspectives. Semin Hematol. 2019;56(4):241-247.
20. Heim KF, Fleisher TA, Stroncek DF, et al. The relationship between alloimmunization and posttransfusion granulocyte survival: experience in a chronic granulomatous disease cohort. Transfusion. 2011;51(6):1154-1162.
21. Arnold DE, Chellapandian D, Parikh S, et al. Granulocyte transfusions in patients with chronic granulomatous disease undergoing hematopoietic cell transplantation or gene therapy. J Clin Immunol. 2022;42(5):1026-1035.