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Polycythemia in an Infant Secondary to Granulocyte Transfusions

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Abstract

Granulocyte transfusions may be useful for neutropenic pediatric patients with refractory bacterial or fungal infections. Many potential adverse sequelae associated with granulocyte transfusions are well recognized, including febrile reactions, fluid overload, alloimmunization, and lung injury. Other potential adverse sequelae, however, are less well known. This case report describes an infant with familial hemophagocytic lymphohistiocytosis (FHL) who developed polycythemia (hemoglobin 10 g/dL to 17.6 g/dL) following four daily transfusions of 20 ml/kg of apheresis collected, steroid stimulated donor granulocytes. Expanded knowledge of potential risks of transfused granulocytes will allow for rapid recognition of transfusion related complications, should they occur.

Keywords

Transfusion; red blood cells; granulocytes; neutropenia

INTRODUCTION

Granulocyte transfusions may be utilized to treat severe bacterial or fungal infections in patients with neutropenia or congenital neutrophil function defects [1]. Although granulocytes have been collected and transfused for years, debate continues regarding their therapeutic utility [2]. The number of granulocytes as well as the degree of contamination with platelets and red blood cells (RBCs) vary significantly with collection methodology and donor treatment. Buffy coat pooling from whole blood donations occurs at some centers [3,4]. Donor priming with dexamethasone, granulocyte colony stimulating factor (G-CSF), or a combination of the two, followed by apheresis collection of granulocytes, occurs at other centers [5–9]. Given that a typical granulocyte product may contain up to 50 mL of contaminating RBCs, products must be ABO and crossmatch compatible per AABB and FDA requirements [10,11].

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The risk/benefit ratio of granulocytes must be carefully considered prior to transfusion. Granulocytes have an extremely short life span (<24 hours), necessitating transfusion soon after collection; thus, the products may be released before infectious disease testing has been completed. Furthermore, granulocytes from cytomegalovirus (CMV) seronegative donors may not always be available. Additionally, transfusion of granulocytes may result in traditional transfusion reactions including febrile, allergic, and hemolytic, as well as circulatory overload, transfusion related acute lung injury (TRALI), and transfusion associated graft versus host disease [1]. Finally, patients may develop anti-HLA antibodies, anti-RBC antibodies, or respiratory difficulties due to WBC trafficking to the lungs [12–14]. Herein, we report another additional potential complication (polycythemia), observed in a neonate following the transfusion of apheresis collected, steroid stimulated donor granulocytes.

CASE REPORT

A previously healthy 10-week old, 4.3 kg infant presented with a one week history of fever, fussiness, and decreased feeding. Physical examination was significant for hepatosplenomegaly. Her initial laboratory values revealed anemia (hemoglobin of 9.6 g/dL), thrombocytopenia (platelet count of 16,000/uL), coagulopathy (PT of 22.7 seconds, upper limit of normal (ULN)=15.9 sec, PTT of 50.5 seconds, ULN=42.1 sec, fibrinogen 78 mg/dL, ULN=394 mg/dL), elevated ferritin (8700 ng/mL, ULN=400 ng/mL, elevated triglycerides (199 mg/dL, ULN=150 mg/dL) and slightly elevated liver enzymes (AST 77 U/L (ULN=60U/L), ALT 82 U/L (ULN=50U/L), GGT 196 U/L (ULN=160 U/L)). Viral testing was negative for acute CMV, HSV, HHV-6, HHV-8, and Parvovirus B-19. She was treated empirically with vancomycin, cefotaxime, acyclovir, and vitamin K, along with blood product support. Elevated CD25/soluble IL-2 receptor (sIL-2R) (9632 units/mL, ULN=3026), absent NK cell activity, the presence of bone marrow and CNS hemophagocytosis, and homozygous perforin gene (PRF1) mutation confirmed the diagnosis of Familial Hemophagocytic Lymphohistiocytosis (FHL).

The patient received dexamethasone, etoposide, cyclosporine, and intrathecal methotrexate. She developed a recurrence of fever 3 weeks after admission while still on broad spectrum antibiotics, and blood cultures grew *Candida lusitanae*. She was initially treated with liposomal amphotericin and then switched to fluconazole; blood cultures remained positive for 2 weeks despite anti-fungal therapy and a trial of granulocyte colony stimulating factor. No evidence of ocular seeding or cardiac involvement was evident; however, urine was positive for yeast and a CT scan of the abdomen showed presumed splenic and gallbladder fungal lesions. Granulocyte transfusions were initiated for protracted neutropenia and refractory fungal sepsis.

Granulocyte donors were recruited from the American Red Cross (ARC) platelet pheresis donor pool and met allogeneic donor screening and testing criteria as defined by the FDA and the AABB. Donors received a total of 6 mg of oral dexamethasone per square meter of body surface area divided in two doses with half the dose taken twelve hours prior to collection and half the dose taken three hours prior to collection. Granulocyte units were collected by continuous-flow apheresis (COBE Spectra cell separator, CaridianBCT, Lakewood, CO) using centrifugation to separate the granulocytes from the blood. The target inlet volume was 7000 mL with a collect rate of 3 mL/min and a separation factor of 250. The collect line where it exited the centrifuge was monitored using the WBC color gram for a desired hematocrit of 7.5% and changes were made in the plasma pump flow rate in 0.3 to 1.0 mL/min increments every 3–5 minutes as needed. Trisodium citrate, 30 mL of 46.7% solution, was added to a 500mL bag of 6% hydroxyethyl starch and the solution was mixed every fifteen minutes during the collection. The citrate-containing HES solution was added

continuously at a ratio of 13:1 with whole blood. The procedure ended when the anticoagulant solution was completely infused or the target endpoint had been reached. Blood counts (Advia 120 Automated hematology Analyzer, Siemens, USA), were performed on samples collected from the donor immediately prior to the donation and from the granulocyte product immediately after collection (Table I).

The patient received a total of 4 granulocyte transfusions over 4 days. The patient's laboratory values prior, during, and following the granulocyte transfusions are shown in Figure 1, with arrows representing the timing of granulocyte transfusions. Of note, her hemoglobin prior to the first granulocyte transfusion was 10 g/dL, with a peak of 17.6 g/dL after the 4th transfusion. Granulocyte transfusions were discontinued after 4 days given the patient's polycythemia. Her fungal sepsis resolved 1 week after initiation of granulocyte therapy, and she subsequently underwent an unrelated, partially matched, allogeneic cord blood transplant. Six months post-transplant she has fully engrafted, is FHL disease free, and is transfusion independent; however, she remains hospitalized with chronic graft versus host disease and feeding issues.

DISCUSSION

This report demonstrates polycythemia in an infant secondary to granulocyte transfusions, an infrequently reported complication. Granulocyte preparations contain relatively large amounts of contaminating RBCs secondary to difficulties in excluding RBCs while retaining functional granulocytes. This RBC contamination of granulocyte products is well known to transfusion medicine physicians, with a need for the products to be ABO and crossmatch compatible due to this contamination. Donor centers are actively investigating methods to decrease RBC contamination of granulocyte products, through the use of hydroxyethyl starch and gravity sedimentation of apheresis products [9], or through modification of pooled buffy coat products to include platelet additive solution for enhanced separation of RBCs from granulocytes [3].

The polycythemia observed in this case was due to a relatively large volume of granulocyte product being transfused into a small infant with a small plasma volume. Such hemoglobin changes would likely be unnoticed in older and larger children who receive dexamethasone stimulated donor granulocytes. This patient received 20 mL/kg of granulocytes daily for 4 days, with a steady rise in hemoglobin noted after the second granulocyte transfusion. Taking the Hct of the transfused units into consideration, in total the patient received an equivalent of 20 mL/kg of RBCs at 60% Hct. Therapeutic phlebotomy was considered for treatment of the patient's polycythemia when the hemoglobin reached 17.6 g/dL, but was ultimately not necessary as she remained stable. Subsequently, the patient did not require another RBC transfusion for 14 days.

There is no specific recommended granulocyte dose for infants or children, with the entire unit, which is typically 200–300 mL, often being too much volume for a child to tolerate. The dose of 20 mL/kg was chosen at the recommendation of the transfusion service, with a goal of maximal granulocyte infusion without volume overload. Optimal dosing for granulocytes per transfusion has been described to be 1.0×10^{10} granulocytes/m² of recipient body surface area per transfusion or 1.4×10^8 granulocytes/kg [2,15,16]; however, the exact minimal effective dose is uncertain [17,18]. Furthermore, dosing intervals are not well established.

The granulocyte products transfused to the described patient were collected from dexamethasone primed donors by continuous flow apheresis, per local ARC protocol. However, it is increasingly being accepted that products from donors primed with G-CSF

(or dexamethasone in combination with G-CSF) have higher granulocyte counts than products from donors primed with dexamethasone alone [8]. More efficient donor priming theoretically equates to a lower product volume transfused per recipient. The most recent Cochran analysis [2] states that “well designed prospective trials are required to evaluate the efficacy of granulocyte transfusions,” with a recommendation to transfuse at least 1.0×10^{10} granulocytes/adult recipient.

In summary, this case highlights a potential adverse outcome of apheresis derived granulocyte transfusions that, to our knowledge, has not previously been described. Although donor centers and transfusion medicine services are aware of RBC contamination of granulocyte products, this complication may not be in a clinician’s differential diagnosis of polycythemia in a child being transfused with granulocytes. Expanded knowledge of the potential risks of transfused granulocytes will allow for clinicians to rapidly recognize transfusion related complications should they occur.

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Figure 1. Patient's laboratory response to transfused granulocytes

Trends of patient's total white blood cell count (A), absolute neutrophil count (B) and hemoglobin (C) prior to, during, and after granulocyte transfusions. The arrows indicate time points of granulocyte transfusions.

Table 1

Laboratory values of granulocyte products and granulocyte donors

	Granulocyte Product Counts				Donor Blood Counts			
	Product 1	Product 2	Product 3	Product 4	Donor 1	Donor 2	Donor 3	Donor 4
WBC ($\times 10^3/\mu\text{L}$)	93.4	76.6	55.3	55.6	8.23	8.93	5.12	6.67
RBC ($\times 10^6/\mu\text{L}$)	1.08	1.9	2.07	1.51	4.94	4.39	4.95	5.19
Hemoglobin (g/dL)	3.0	6.1	6.3	3.6	14.6	14.3	15.3	13.3
Hematocrit (%)	9.5	18.1	18.9	11.8	42.1	41.7	44.4	40.4