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Infusion Hemolysis after Pediatric Major ABO Mismatched BMT: Comparison of Two Red Blood Cell Depletion Techniques

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Abstract

Background—During major ABO mismatched bone marrow transplant (BMT) the infusion of incompatible red blood cells (RBCs) that are present in the bone marrow graft can cause adverse events from hemolysis. RBC depletion of the bone marrow graft can decrease this risk, but the optimal method to prevent hemolysis is unclear.

Procedure—We conducted a retrospective cohort study of patients who underwent major ABO mismatched BMT at a pediatric center and had RBC depletion with either hydroxyethyl starch (HES) sedimentation or Ficoll density gradient separation. Post-infusion hemoglobinuria and creatinine values were compared.

Results—Between 2002 and 2016, 37 patients received HES-treated and 16 patients received Ficoll-treated major ABO mismatched bone marrow grafts. The median residual volume of RBCs was significantly greater with HES-treated grafts (HES 21.0 ml vs. Ficoll 1.4 ml, p<0.0001). Patients who received HES-treated grafts had a higher prevalence of post-infusion hemoglobinuria (HES 57% vs. Ficoll 6%, p=0.0009), but renal impairment was rare. Considering only HES-treated grafts, the volume of RBCs was not associated with either post-infusion hemoglobinuria or a creatinine increase.

Conclusions—Ficoll density gradient separation achieves smaller RBC volumes and less post-infusion hemoglobinuria than HES sedimentation, but both can prevent significant hemolysis. Further studies are needed to determine the residual incompatible RBC volume threshold in major ABO mismatched BMT.

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Keywords

ABO; major mismatch; incompatible; hemolysis; pediatric BMT

INTRODUCTION

ABO incompatibility between donor and recipient in bone marrow transplantation (BMT) is common. BMT donors are selected primarily on the basis of the human leukocyte antigen complex which is inherited independently of the ABO blood group system. ABO incompatible BMTs are characterized as: major mismatch when the recipient has anti-A and/or anti-B isohemagglutinins against donor (A→O, B→O, AB→O, AB→A, AB→ B), minor mismatch when the donor has isohemagglutinins against recipient (O→A, O→B, O→AB, A→AB, B→AB), or bidirectional mismatch when both are present (A→B, B→A). The impact of ABO incompatibility on survival after allogeneic hematopoietic stem cell transplantation is controversial with some studies finding no association (1–8), and other studies linking ABO incompatibility with increased mortality (9–15). Nonetheless, major ABO incompatibility has been consistently associated with increased transfusion requirements and the rare complication pure RBC aplasia after transplant (3–5, 9, 12, 13).

Another specific potential complication of major ABO mismatched BMT is acute hemolysis of incompatible red blood cells (RBCs) present in the bone marrow graft. This hemolysis can cause renal dysfunction and, if severe, lead to fatal multi-organ failure (16, 17). To prevent this infusion complication, two general approaches exist: the BMT recipient's isohemagglutinin titers can be decreased, or the amount of donor RBCs in the bone marrow graft can be decreased. While recipient isohemagglutinin reduction with plasma exchange pre-BMT has been shown to prevent clinically significant infusion-related hemolysis, this approach requires recipients to undergo plasma exchange and can fail to achieve a desired reduction in the isohemagglutinin titer even after multiple exchanges (18). The alternative approach, graft manipulation to decrease the amount of incompatible RBCs, can be accomplished with various different RBC depletion techniques including hydroxyethyl starch (HES) sedimentation, Ficoll density gradient separation, and blood cell separator processing (19–23).

Evidence-based guidelines regarding the prevention of major ABO mismatched BMT infusion hemolysis do not exist. Regarding RBC depletion in particular, transplant centers employ different methods and have varied standards with regard to the amount of residual incompatible RBCs that can be infused with the bone marrow graft (24). This issue is potentially more important in pediatrics, where a small absolute volume of incompatible RBCs could still constitute a significant volume per body weight of the recipient. The Foundation for the Accreditation of Cellular Therapy mandates that institutions should have a "policy for volume of ABO-incompatible red cells in allogeneic cellular therapy products," without mention of a specific volume target (25). Given this lack of clarity regarding the optimal RBC depletion method, we sought to directly compare hemolysis post major ABO mismatched BMT infusion between children who underwent RBC depletion with HES

sedimentation versus Ficoll density gradient separation. We also sought to characterize the volume of residual incompatible RBCs associated with this hemolysis.

METHODS

Study Design

We conducted a retrospective cohort study of all patients who received a major ABO mismatched BMT at a single pediatric institution between 2002 and 2016. Patients who received a bidirectional mismatch BMT were excluded for this analysis. Approval for this study was obtained by the Children's Healthcare of Atlanta Institutional Review Board.

Institutional Practice for Major ABO Mismatched Transplants

All major ABO mismatched BMT patients during this time received similar supportive care with the bone marrow infusion that included premedication with acetaminophen 10–15 mg/kg, diphenhydramine 1 mg/kg, and hydrocortisone 4 mg/kg or methylprednisolone 1–2 mg/kg. All patients received intravenous hyperhydration at 1.5–2 times maintenance starting 4 hours prior to the infusion, continued until two urine tests negative for blood were obtained post infusion.

RBC Depletion Techniques

Prior to June 2014, RBC depletion with HES sedimentation was performed on most major ABO mismatched BMTs. After June 2014, RBC depletion with Ficoll density gradient separation was exclusively used for major ABO mismatched BMTs. HES RBC depletion was achieved by diluting the hematocrit of the bone marrow product to 25% with saline/albumin, followed by adding one part Hespan® to seven parts diluted product, which caused RBC aggregation and rouleaux formation leading to RBC sedimentation. The sedimented RBCs were then drained and a variable volume of residual RBCs remained to avoid the loss of hematopoietic progenitor cells. Ficoll RBC depletion was performed by adding 30 ml aliquots of marrow diluted 1:1 with saline/diluent to a variable number of conical tubes containing 15 ml of Ficoll-Paque. These tubes were then centrifuged for 30 minutes at 1500 rpm at 20°C to create a mononuclear cell layer containing hematopoietic progenitor cells devoid of RBCs and granulocytes.

Isohemagglutinin titers

Patients with a banked pre-BMT plasma sample available for study had IgG isohemagglutinin titers measured using the conventional test tube method. Briefly, a 2% suspension of the target RBCs were incubated with each plasma dilution for 1 hour at 37°C, followed by a $4 \times$ wash in saline and subsequent incubation with anti-human globulin IgG. Cells were then centrifuged, followed by examination for agglutination. All testing was performed in parallel using the standard clinical protocols employed by the Emory University Hospital Blood Bank.

Clinical evidence of hemolysis

As a marker of potential hemolysis-induced renal dysfunction, serum creatinine the day after the bone marrow infusion (day +1) was compared to the creatinine the day of the infusion (day 0) to calculate a post/pre creatinine ratio. As an additional marker of hemolysis, post-infusion urine dipstick results were available for review on all patients transplanted after 2006. Urine positive for blood was used to define hemoglobinuria.

Statistical Analysis

Differences in categorical data were analyzed using the chi-square test or Fisher exact test. Differences in continuous data were analyzed using the Wilcoxon rank-sum test. Statistical calculations were performed with SAS 9.3 (SAS Institute Inc., Cary, NC). Graphics were created using GraphPad Prism version 7.02 (GraphPad Software, La Jolla, CA).

RESULTS

Patient Characteristics

Fifty-three patients underwent major ABO mismatched BMT between October 2002 and September 2016. Patient diagnoses included: acute myeloid leukemia (n=9), acute lymphoblastic leukemia (n=8), mixed phenotype leukemia (n=2), myelodysplastic syndrome (n=2), other malignancies (n=2), aplastic anemia (n=8), thalassemia (n=5), sickle cell disease (n=4), Fanconi anemia (n=3), Wiskott-Aldrich syndrome (n=2), and other nonmalignant conditions (n=8). Median patient age was 9.9 years (range 0.5–22.9 years). Median patient weight was 30.7 kg (range 6.7–131.7 kg). No patients had pre-BMT plasmapheresis to reduce isohemagglutinin titers. RBC depletion of the bone marrow graft was performed with HES for 37 patients and with Ficoll for 16 patients. Patient characteristics of these two groups were similar with the exception that more patients whose grafts underwent RBC depletion with HES had a diagnosis of a malignancy (Table 1).

Comparison of Red Blood Cell Depletion with HES versus Ficoll

The volume of RBCs remaining after RBC depletion with HES was significantly greater than after Ficoll (Table 2). Hemoglobinuria after the bone marrow infusion was significantly more common for patients with HES-treated grafts (Table 3). This difference remained significant when excluding patients with a malignancy diagnosis: hemoglobinuria present in HES group 8/15 (53%) versus Ficoll group 1/13 (8%), p=0.02. For the 17 patients with a post-infusion urine positive for blood, all patients had two subsequent urine dipstick results negative for blood in less than 24 hours with the exception of the single Ficoll patient and the one patient who had clinically significant renal impairment (noted below). More patients with HES-treated grafts also had a greater than 50% post-infusion rise in their creatinine, but the median creatinine ratios were similar (Table 3). Only a single patient was noted to have overt renal impairment after the bone marrow infusion requiring changes to the clinical management. This patient received a bone marrow graft that contained 51 ml (1.1 ml/kg) of residual incompatible RBCs after treatment with HES. The details of this patient's clinical course have been previously reported (16). All patients had donor engraftment with a similar

time to neutrophil engraftment for both groups: HES median day +20 versus Ficoll median day +19.5, p=0.56.

Risk Factors for Evidence of Hemolysis among Patients who Received HES-treated Grafts

Among patients who received HES-treated grafts, patients with hemoglobinuria had a significantly greater increase in their creatinine ratio compared to patients with no hemoglobinuria (median 1.26 vs. 1.00, p=0.003), and all patients with a greater than 50% creatinine increase had hemoglobinuria. The median volume of incompatible RBCs infused was not significantly different between patients with hemoglobinuria: 24.5 ml (range 4.5 – 65 ml), 0.74 ml/kg (range 0.23 – 2.8 ml/kg); and with no hemoglobinuria: 20.5 ml (range 2.7 – 43 ml), 0.64 ml/kg (range 0.12 – 1.4 ml/kg), (p= 0.24, 0.63). The total volume of residual RBCs after HES-treatment and volume per patient weight was also not significantly associated with the creatinine ratio (Fig. 1). Pre-BMT isohemagglutinin titers were measured for 9 patients who received HES-treated grafts (Supplemental Table S1). All 4 patients (100%) with an anti-A or anti-B against donor at high titer (1:32) had hemoglobinuria, compared to 3/5 (60%) patients with a low isohemagglutinin titer (1:16), p=0.44. The creatinine ratio was similar for these two groups (high titer 1.14 vs. low titer 1.00, p=0.99). Pre-BMT GFR was not associated with the creatinine ratio when analyzed as a continuous variable (Supplemental Figure S1) or as a dichotomous variable (abnormal GFR group median ratio 1.0 vs. normal GFR group median ratio 1.0, p=0.30).

DISCUSSION

We directly compared for the first time RBC depletion using two different techniques for pediatric major ABO mismatched BMT. Compared to the HES sedimentation method, RBC depletion with Ficoll density gradient separation achieved smaller residual incompatible RBC volumes and resulted in less evidence of infusion hemolysis. While bone marrow graft manipulation could affect hematopoietic progenitor cells, we also importantly found that engraftment was similar with these two techniques.

Infusion-related hemolysis was more common with the HES sedimentation technique likely due to the larger volume of RBCs remaining in the graft, but almost all patients who received HES-treated grafts did not have major adverse events from the infusion of residual incompatible RBCs. We thus sought to further analyze this group to determine if a certain amount of RBCs was associated with evidence of hemolysis-induced renal dysfunction. Unexpectedly, we found that both the total volume and volume per patient weight of incompatible RBCs were not significantly associated with either post-infusion hemoglobinuria or an increased creatinine. In seeking to determine what residual volume of incompatible RBCs could safely be infused following RBC depletion in pediatric major ABO mismatched BMT, Patrick et al similarly found no significant difference in the mean volume of incompatible RBCs given to those patients who had any minor hemolytic or clinically significant adverse event compared with those who did not (26). These findings suggest that there is no simple, single "safe" volume or volume per patient weight standard of incompatible RBCs. Instead, it appears that when the incompatible RBC volume is

reduced to the range of 5 to 60 ml or 0.2 to 2 ml/kg, variables other than the RBC amount significantly impact which patients will have clinical evidence of infusion hemolysis.

One other variable suspected to influence the amount of hemolysis after infusion of ABO incompatible RBCs is recipient isohemagglutinin titer. All studied patients with a high isohemagglutinin titer had post-infusion hemoglobinuria, but the small number of patients who had isohemagglutinin titer testing as part of this study prevent us from drawing any conclusions about the significance of this variable. Future studies should further investigate if isohemagglutinin titers influence hemolysis in this setting.

This study contributes to the limited literature on infusion hemolysis after major ABO mismatched BMT with our comparison of two RBC depletion techniques, but limitations exist. We only studied patients transplanted at a single center, potentially limiting the generalizability of our findings. This study design, however, minimized confounding variables since all patients received similar supportive care. The use of the two different RBC depletion techniques was due to a change of practice at this center over time; nonetheless, other practices associated with the bone marrow infusion did not change during this time. Fewer patients had a malignancy diagnosis in the Ficoll group (group transplanted more recently) due to the increasing proportion of BMTs for nonmalignant indications that has occurred at this center over time.

It is possible that a post-infusion urine dipstick positive for blood result was not due to hemoglobinuria caused by the infusion, or that an increased post-infusion creatinine was not from hemolysis-induced renal dysfunction. Yet, our finding that patients with positive urine results had higher creatinine ratios supports the idea that these markers complement each other and reflect hemolysis. Since microscopic examination of the urine was not routinely performed, it is also possible that a urine dipstick positive for blood represented hematuria (presence of red blood cells) rather than hemoglobinuria (presence of free hemoglobin). Nonetheless, the fact that patients with urine positive for blood immediately after the infusion subsequently had clear urine is consistent with the positive urine results reflecting hemoglobinuria from hemolysis of incompatible RBCs. While this study did not include serial monitoring of lactate dehydrogenase or indirect bilirubin that may represent more sensitive markers of hemolysis, our studied outcomes (hemoglobinuria and post/pre creatinine ratio) likely are more clinically relevant markers of hemolysis as they directly concern renal dysfunction that is the feared clinical complication in this setting. Finally, it is also possible that hemolysis of donor RBCs remaining in the graft could have occurred prior to graft infusion. We thus cannot definitively conclude that post-infusion hemolysis was due to recipient isohemagglutinins without an ABO compatible BMT control group.

RBC depletion can cause loss of hematopoietic progenitor cells that could affect engraftment. We could not compare this loss from the two different techniques by measuring the change in the total nucleated cell count before and after RBC depletion because of the different composition of the products after processing. Granulocytes are removed from the bone marrow product with Ficoll density gradient separation, but they remain after HES. Our finding that time to neutrophil engraftment was similar for both Ficoll and HES groups

suggests that clinically significant differences in hematopoietic progenitor cell losses do not occur after these two methods.

RBC depletion using HES sedimentation and Ficoll density gradient separation are both effective means to avoid clinically significant hemolysis associated with the infusion of incompatible RBCs in pediatric major ABO mismatched BMT. RBC depletion with HES sedimentation does result in a larger volume of RBCs remaining in the bone marrow graft that could cause infusion hemolysis, but it remains unclear what residual amount of incompatible RBCs is safe. Given this uncertainty, this center currently continues to employ RBC depletion with Ficoll density gradient separation for all major ABO mismatched BMTs. RBC depletion with Ficoll density gradient separation is more labor intensive for laboratory staff than HES sedimentation, so it may not be chosen by or even possible at many centers. Further research is needed to explore the economics in addition to the safety of RBC depletion methods for major ABO mismatched BMT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

BMT	bone marrow transplant
RBC	red blood cell
HES	hydroxyethyl starch
IQR	interquartile range
GFR	glomerular filtration rate

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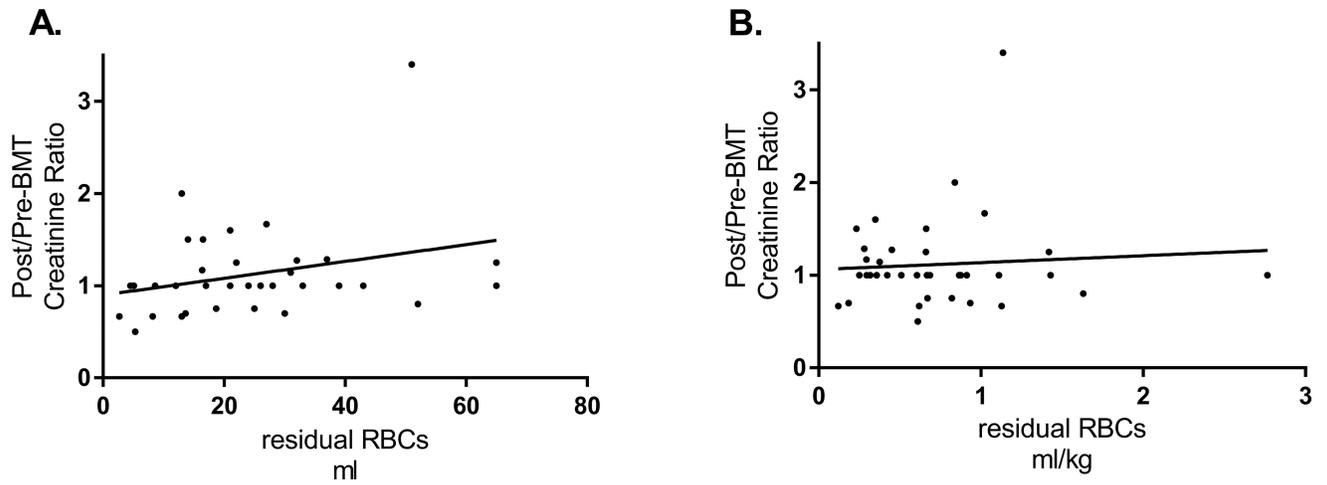
FIGURE 1.

Relationship between Amount of Residual Incompatible Red Blood Cells after Hydroxyethyl Starch Depletion and Creatinine Ratio

A. Total Volume. Line represents line of best fit from linear regression analysis: post/pre-BMT creatinine = 0.0091 (volume) + 0.90, r^2=0.087, slope p=0.08

B. Volume per Patient Weight. Line represents line of best fit from linear regression analysis: post/pre-BMT creatinine = 0.075 (volume/patient weight) + 1.06, r^2=0.006, slope p=0.65

TABLE 1

Demographic and Clinical Characteristics of Patients

	HES (n=37)	Ficoll (n=16)	p-value
Age, median (IQR)	10.0 years (7.3)	9.2 years (9.1)	0.75
Weight kg, median (IQR)	32.1 (34.5)	28.4 (27.6)	0.27
Male, number (%)	24 (65%)	12 (75%)	0.47
Diagnosis of malignancy, number (%)	20 (54%)	3 (19%)	0.017
Myeloablative conditioning, number (%)	21 (57%)	6 (38%)	0.20
Abnormal Pre-BMT GFR, [*] number (%)	11 (30%)	5 (31%)	0.91
Type of ABO mismatch, number (%)			
A→O	21 (57%)	10 (63%)	0.75
B→O	10 (27%)	6 (38%)	
AB→O	1 (3%)	0	
AB→A	3 (8%)	0	
AB→B	2 (5%)	0	

HES, hydroxyethyl starch. IQR, interquartile range. GFR, glomerular filtration rate.

[*] Abnormal GFR defined as defined as <90 ml/min/1.73m^2 for patients age >2 years, <65 ml/min/1.73m^2 for patients age 1–2 years, and <50 ml/min/1.73m^2 for patients age <1 year.

TABLE 2

Comparison of Red Blood Cell Volumes in Hydroxyethyl Starch versus Ficoll-treated Grafts

	HES	Ficoll	p-value
Pre-Depletion RBC Volume, median (IQR)	285 ml (168)	218 ml (140)	0.06
Post-Depletion RBC volume, median (IQR)	21.0 ml (18)	1.4 ml (1.1)	<0.0001
Post-Depletion RBC volume/patient weight, median (IQR)	0.66 ml/kg (0.56)	0.05 ml/kg (0.09)	<0.0001

HES, hydroxyethyl starch. IQR, interquartile range.

TABLE 3

Comparison of Markers of Hemolysis in Patients who received Grafts that Underwent Red Blood Cell Depletion using Hydroxyethyl Starch versus Ficoll

	HES	Ficoll	p-value
Hemoglobinuria, [*] number (%)	16/28[†] (57%)	1/16[†] (6%)	0.0009
>50% Rise in Creatinine, number (%)	4/37 (11%)	0/16 (0%)	0.30
Post/Pre-BMT Creatinine Ratio, median (IQR)	1.0 (0.3)	1.0 (0.1)	0.70

HES, hydroxyethyl starch.

[*] Hemoglobinuria defined by the presence of blood on urine dipstick.

[†] Urine dipstick results only available after 2006 for review, 9 patients were excluded from this analysis.