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BRIEF REPORT

APHERESIS RBC ASSOCIATED WITH REPEATED HEMOLYSIS DURING BLOOD PRIMING OF THE CELLEX PHOTOPHERESIS SYSTEM.

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ABSTRACT

Extracorporeal photopheresis (ECP) in young pediatric patients has a risk for procedural hypotension and anemia due to extracorporeal fluid shifts. A standard mitigation policy in these patients is to prime the device with packed red blood cells (RBC) or whole blood. We now report multiple episodes of hemolysis while attempting to prime the Therakos™ Cellex™ in a pediatric transplant patient undergoing a course of ECP for severe graft-versus-host-disease. Over the course of 40 ECP treatments, hemolysis was observed on five occasions. An extensive investigation found an association between hemolysis and apheresis RBC (A-RBC). Of 46 RBC units dispensed for blood priming, hemolysis occurred with 22% (4/18) of A-RBC and accounted for 80% (4/5) of all hemolysis episodes. Hemolysis was significantly higher with A-RBC when compared to RBC collected by whole blood donations (WB-RBC: 3.5% [1/28]; p=0.049). A comparison of RBC attributes, including unit age, showed that hemolyzed A-RBC units tended to be younger than both non-hemolyzed RBC (6.5 vs 10.3 days, p=0.018) and WB-RBC (8.5 days, p=0.10). We hypothesize that A-RBC may exhibit "sublethal" RBC damage following prior exposure to centrifugal shear and negative forces at the time collection, leading to a decrease in RBC deformability and increased susceptibility to hemolysis. This is the first report showing an increased susceptibility to hemolysis with A-RBC during priming of the Cellex™.

Brief Report

Extracorporeal photophoresis (ECP) in small children requires additional care to prevent procedural hypotension due to small blood volumes, discontinuous flow and large extracorporeal volumes.[1] Several approaches are used in pediatric patients including optimizing the pre-procedure hematocrit, intra-procedure fluid boluses, and/or priming the ECP device with packed RBC or whole blood.[1] We now report repeated episodes of hemolysis during RBC priming of the Therakos™Cellex™ Photopheresis system (Mallinckrodt Pharmaceuticals, St. Louis, MO) in a pediatric patient.

The patient was a 7.5-year-old, 30 kg male with high-risk B-cell ALL, who underwent myeloablative conditioning with fludarabine, thymoglobulin and busulfan, followed by an 8/8 HLA-matched, unrelated, ABO-incompatible (patient A+/donor O-) allogeneic single cord transplant. His post-transplant graft-versus-host-disease (GVHD) prophylaxis included tacrolimus and mycophenolate. On day +30 post-transplant, the patient developed a cough and new mildly pruritic, papular rash on lower legs and hands. By day +33, the rash had extended to include legs, arms, palms and face involving 45-50% of body surface area (BSA), consistent with stage 2 cutaneous GVHD. The patient rapidly progressed to stage 4 cutaneous GVHD despite topical and IV steriods and was started on a 4 week course of basliximab (day +38) and 20 week course of ECP. Due to medical issues, including severe hypertension, the start of ECP treatment was delayed until day +50.

ECP was performed on a Cellex using citrate anticoagulation and a 12 Fr trifusion Hickman catheter for venous access. Due to the patient's small size, a RBC prime was performed following the manufacturer's instructions. At the onset of the second scheduled ECP treatment, pink-tinged plasma was noted during blood priming, consistent with hemolysis. The procedure was reinitiated with a second RBC unit and was completed without difficulty (Table 1). Hemolysis recurred while priming for ECP#9 (2/22/18). A second RBC unit was again provided and the procedure was successfully completed. Three episodes of hemolysis occurred while attempting to complete ECP#40 (6/26/18 – 7/10/18). In one instance, the device was successfully re-primed with a new RBC unit, although the procedure was ultimately cancelled due to unrelated access issues. Hemolysis during priming was again noted in the next two scheduled ECP with cancellation of those procedures. The patient successfully completed a course of 40 ECP in July 2018.

An investigation was initiated by the photopheresis and transfusion medicine services to identify any factors associated with hemolysis. There was no relationship between hemolysis and a specific ECP device or kit lot numbers over the 5.5-month period. There was no evidence of intra-procedure hemolysis associated with any ECP. There was a single episode of a high system pressure alarm (6/26/18) associated with RBC priming and hemolysis. The device was successfully re-primed with another RBC unit without recurrence of hemolysis.

A total of 46 RBC were dispensed and included both apheresis (A-RBC, n=18) and whole blood-derived RBC (WB-RBC, n=28). All RBC were group O-, prestorage leukoreduced in additive solution (AS1, AS3; mean Hct=60%)[3], and were irradiated immediately prior to dispensing to the floor. Most RBC (89%) were less ≤ 14 days of age (median, 8 days). The majority (4/5, 80%) of hemolyzed units were A-RBC, with an average age of 6.8 ± 2 days (range 4-9 days, Table 1). In contrast, only one, older WB-RBC hemolyzed early in ECP treatment (ECP#2, unit age 33 days). Overall, A-RBC were significantly more likely than WB-RBC to hemolyze during blood prime (22% vs 3.5% units, p=0.049). Hemolyzed A-RBC tended to be fresher than both non-hemolyzed A-RBC (10.8 ± 5.2 days, p=0.018) and WB-RBC (8.5 ± 3 days, p=0.10). Based on blood supplier and unit volume, A-RBC were collected using the Fenwal Alyx™ collection system (Fresenius Kabi, Lake Zurich, IL).

A MedWatch review identified 22 reports that contained both Cellex and hemolysis in the narrative.[2] Hemolysis was observed during or after ECP in 12 reports, including three pediatric patients (Supplement Table S1). Causes of hemolysis included excessive heat and mechanical shear due to device malfunction, small caliber CVC and clots.[2,4] To our knowledge, this is the first report indicating an increased risk of hemolysis with A-RBC during blood priming of the Cellex.

We hypothesize that A-RBC may exhibit “sublethal” RBC damage following prior exposure to centrifugal shear and negative pressures during collection.[5] Sublethal

RBC damage is a recognized phenomenon in extracorporeal circuits and is associated with a decrease in red cell deformability and increased susceptibility to hemolysis, even after exposure to relatively modest shear forces.[5,6] Sublethal RBC damage, with a 3-fold increase in mechanical fragility, is also observed after washing RBC with the COBE 2991 cell processor.[7] Likewise, Fenwal Alyx A-RBC are reported to have higher free hemoglobin, lower ATP content and increased membrane rigidity at the time of collection when compared to WB-RBC.[8,9] Irradiation may have further increased RBC rigidity: Irradiation has been shown to immediately reduce RBC elongation and deformability within minutes of irradiation, even in RBC $\leq$ 7 days of age.[10]

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Table 1. Summary of Hemolysis during RBC Blood Prime of the Therakos™Cellex™ Photopheresis System

Date	ECP No.	Procedure Completed (Yes/No)	ECP Equipment		Packed RBC Dispensed for Blood Prime[a]				Hemolysis (Yes/No)
			Device No.	Kit Lot No.	RBC[b,c] (A/WB)	Unit Age (days)	Additive Solution	Unit Volume (mL)	
1/31/18	2	Y	40257	F359	WB-RBC	33	AS1	350	Y
			40257	F359	WB-RBC	8	AS1	350	N
2/22/18	9	Y	40257	F367	A-RBC	9	AS3	286	Y
			40257	F367	WB-RBC	9	AS3	350	N
6/26/18	40	N[d]	40158	G319	A-RBC	5	AS3	272	Y
			40158	G319	WB-RBC		AS1	350	N
6/27/18	40	N	40158	G319	A-RBC	6	AS3	281	Y
7/10/18	40	N	40158	G318	A-RBC	4	AS3	292	Y
7/11/18	40	Y	40158	G318	A-RBC	5	AS3	281	N

a. All RBC were group O-, pre-storaged leukoreduced and irradiated immediately prior to release.

b. A-RBC, apheresis RBC; WB-RBC, RBC processed from whole blood donations.

c. The average hematocrit (%) of both A-RBC and WB-RBC stored in AS is 60%.[3]

d. The 6/26/18 procedure was cancelled due to time constraints and unrelated access issues.