Published in final edited form as:

J Pediatr Hematol Oncol. 2015 March ; 37(2): e135–e137. doi:10.1097/MPH.0000000000000241.

Severe hemolytic transfusion reaction due to anti-D in a D+ patient with sickle cell disease

Tina S. Ipe, MD¹, Jennifer J. Wilkes, MD², Helge D. Hartung, MD², Connie M. Westhoff, PhD³, Stella T. Chou, MD^{2,*}, and David F. Friedman, MD^{2,4}

¹Department of Pathology, Houston Methodist Hospital, Houston TX

²Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia PA

³Immunohematology and Genomics Laboratory, New York Blood Center, New York City, NY

⁴Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia PA

Abstract

A 5-year-old male with sickle cell disease presented with pain, dark urine, and fatigue 10 days after a red blood cell (RBC) transfusion. Laboratory evaluation demonstrated severe anemia, blood type O+, and anti-D in the serum. Anti-D in a D+ patient led to *RH* genotyping which revealed homozygosity for *RHD*DAU4* that encodes partial D antigen. Anti-D in this patient whose RBCs exclusively express partial D caused a delayed hemolytic transfusion reaction after exposure to D+ RBCs. The finding of anti-D in a D+ patient should be investigated by molecular methods to help distinguish an alloantibody from an autoantibody.

Keywords

Sickle cell disease; Red blood cell transfusion; Alloimmunization; Delayed hemolytic transfusion reaction; RH genotype

Introduction

Red blood cell (RBC) transfusions are vital to the management of patients with sickle cell disease (SCD) [1]. However, alloimmunization and delayed hemolytic transfusion reactions (DHTRs) remain prevalent in patients with SCD despite RBC matching for the most immunogenic antigens (D, C, E, and K) [2-4]. Recent studies demonstrate that inheritance of variant *RHD* and *RHCE* genes encoding amino acid changes in the Rh antigens (D, C, E, c, and e) is common in individuals of African descent and contributes to alloimmunization in patients with SCD despite Rh-matching programs [2,3,5]. Many *RHD* variants encode partial D proteins that lack some D epitopes and are associated with a risk of alloanti-D formation after exposure to D+ RBCs. We report a 5-year-old male with SCD who typed as

*Corresponding author: Stella T. Chou, 3615 Civic Center Boulevard, ARC 316D, Philadelphia, PA 19104, TEL: 215-590-0947, FAX: 267-426-5476, chous@email.chop.edu.

Author disclosures: The authors have no conflicts of interest to disclose.

D+ but developed a severe DHTR due to an anti-D following transfusion of one unit of D+ RBCs. High-resolution *RH* genotyping of the patient revealed homozygosity for *RHD*DAU4*, a known partial D allele.

Case Report

A 5-year-old boy with SCD, genotype SS, presented to the emergency department with bilateral leg and abdominal pain, dark urine, and fatigue. Ten days prior, he had received one unit of O+ RBCs that was also C, E, and K negative consistent with the institutional antigen matching protocol for patients with SCD who type negative for those antigens. At that time, the antibody screen was negative (Table 1). The patient was transfused 15 cc/kg PRBCs to raise his pre-transfusion hemoglobin of 6.5 gm/dl to an anticipated 9.5 gm/dl, but a hemoglobin level was not measured post-transfusion.

On arrival to the emergency room, his temperature was 39°C; pulse 145 beats/min; respiratory rate 44 breaths/min; and blood pressure 137/86 mmHg. His sclerae were icteric, lungs were clear, and a systolic flow murmur was present. His abdomen showed no hepatosplenomegaly. He appeared fatigued, but his neurologic exam was nonfocal. Laboratory evaluation revealed a hemoglobin of 5.6 gm/dL, reticulocyte count 12.8% (Figure 1A), unconjugated bilirubin 5.6 mg/dL (normal range, 0.2 – 1.0 mg/dL), lactase dehydrogenase 7820 U/L (normal range, 470 – 900 U/L). The urinalysis was positive for large blood, but only 3-5 RBCs per high-power-field were identified indicating hemoglobinuria was present. The patient's serum was icteric (Figure 1B). Hemoglobin quantification performed by automatic gel electrophoresis and densitometric analysis of the bands showed 12% hemoglobin A, 81.7% S, 2.2% F, and 4.1% A₂. The blood type was confirmed as O+ and the antibody screen was weakly positive in a gel-based assay but negative with tube method. A selected cell panel using the gel method detected 1+ reactivity in the patient's serum with anti-D specificity. The direct antiglobulin test (DAT) was negative.

Due to concerns for ongoing hemolysis and worsening anemia, the patient was transferred to the intensive care unit. Twelve hours after presentation, the hemoglobin level declined to 4.8 gm/dL. High-dose methylprednisolone (1 mg/kg every 6 hours for 2 days with subsequent wean) and intravenous immunoglobulin (IVIg, 1 mg/kg once) therapy was initiated. Crossmatch-compatible RBC units negative for D, C, E, and K antigens were prepared for the patient. However, he was not transfused due to concern for hyperhemolysis, an immune phenomenon in patients with SCD whose post-transfusion hemoglobin level is lower than the pre-transfusion value suggesting destruction of both the patient's own RBCs and transfused RBCs [6]. The hemoglobin reached a nadir of 4.2 gm/dL on hospital day 1 and then steadily increased (Figure 1A). The hemoglobinuria improved within 24 hours (Figure 1C). Hemoglobin A was undetectable 16 days after transfusion (Figure 1D). Anti-D reactivity became stronger in subsequent specimens (Table 1).

Given the paradoxical finding of anti-D in a patient whose RBCs type RhD+, high-resolution *RH* genotyping was performed (Molecular Immunohematology Laboratory, NY Blood Center) using automated RHD and RHCE Beadchip DNA array prototypes (Bioarray,

Warren, NJ) and targeted allele-specific polymerase chain reaction (AS-PCR), as described in [2]. DNA-based typing revealed homozygous *RHD*DAU4* and *RHCE*ce48C* alleles, which encode partial D and weak e antigens, respectively.

In retrospect, blood bank records showed one prior transfusion with a type O, D- unit two years earlier. Notably, the patient's first blood bank specimen demonstrated 2+ agglutination in a gel-based typing assay with anti-D reagent, resulting in D+ typing (0.8% Surgiscreen screening cells in the ID-Micro Typing System, Ortho Clinical Diagnostics, Rochester, NY). Typically, D+ individuals react strongly (3 – 4+ agglutination) with standard monoclonal anti-D reagents. The patient's D typing with a less sensitive low ionic strength based agglutination assay was positive only with anti-human globulin (AHG, Ortho Clinical Diagnostics). Two subsequent D typings by gel assay showed 1+ agglutination, suggestive of a weak or partial D phenotype.

Discussion

This case highlights an under-recognized risk factor for Rh alloimmunization in patients with SCD. In the era of C, E, and K matching, the prevalence of RBC alloimmunization ranges from 7% up to 58% in transfused populations with SCD [2-5,7]. Patients with SCD continue to form antibodies against the Rh system (D, C, c, E, e) despite antigen matching programs, underscoring the need to identify partial Rh variants and to distinguish allo- from auto-antibodies. Anti-D produced by D+ patients often represent alloantibodies in individuals with partial D phenotypes, rather than autoantibodies [2,5,8].

This D+ patient had a severe DHTR associated with anti-D following exposure to a single D + RBC unit. Genetic analysis demonstrated homozygous *RHD*DAU4* alleles, predicting exclusive expression of a partial D antigen that differs at two single nucleotide changes that encode two amino acid differences from wild type D antigen [8]. This strongly suggests that the anti-D was an alloantibody formed after transfusion with D+ RBCs that express D epitopes the patient lacks, and should be managed with D- RBCs for all future transfusions. Since the patient's hemoglobin level fell below the pre-transfusion value, suggesting possible hyperhemolysis with clearance of both donor and recipient RBCs [6,9], immunosuppressive therapy was initiated, and transfusion avoided. However, given the severe anemia, antibody identification and availability of crossmatch compatible RBCs lacking D, C, E, and K was expedited.

RBCs with partial D usually type as D+ with commercial serologic reagents; however, depending on reagent and technique, reaction strength may be normal, weak, or positive only with AHG reagent. The majority of partial D alleles likely arose by gene conversion in which parts of the *RHD* gene were substituted by homologous portions of *RHCE*, resulting in loss of some D epitopes as well as formation of novel epitopes. In contrast, patients with classic weak D phenotypes have diminished D antigen density on their RBCs but are not missing D epitopes and are not at risk for D sensitization. The exact frequency of partial D phenotypes in individuals of African descent is not established but appears significantly higher than the <1% frequency in Europeans [2,3,5,10,11]. The most common partial D alleles found in African-Americans are *DAU*, *DIIIa*, and *DIVa*.

Patients with alloanti-D should receive D- RBCs for all subsequent transfusions, even if the anti-D becomes undetectable later. There is no current consensus to provide D- units to all patients with partial D phenotypes to prevent anti-D immunization. In a large cohort of patients with SCD, eleven distinct *RHD* variants that encode altered D antigens were observed [2]. Silvy et al further demonstrated that 18% of patients with SCD and partial D phenotypes developed anti-D, compared to 3% of patients with wild type D [5]. However, since many partial D antigens are found in individuals of African descent, neither study was large enough to correlate specific *RHD* alleles with anti-D formation. Determining which individual *RHD* alleles pose risk of anti-D and DHTRs is a priority and will require larger, multi-institutional studies or registries. Providing D- RBCs to all patients with SCD and partial D may be a strain to the limited D- blood supply nationally. Furthermore, since D- RBCs are more commonly found in donors of European descent, such a strategy may result in an increased incidence of alloantibodies to other RBC antigens with disparate distribution between Europeans and Africans, such as Jk^b, Fy^a, and S. Of note, our patient later formed anti-Jk^b after receiving a subsequent D- RBC unit; for future transfusions, he will receive extended matched RBCs for additional antigens (Fy^a, S) that his RBCs lack. Thus, transfusion support of patients with partial D phenotypes should be individualized with consideration of *RHD* and *RHCE* genotypes, as well as the patient's extended RBC phenotype and alloimmunization history.

In summary, anti-D alloimmunization in patients with SCD can be associated with partial D phenotypes [2,3,5]. Molecular testing should be considered for D+ transfusion recipients who form anti-D and for patients receiving chronic transfusions whose RBCs react weakly with D typing reagents. Partial Rh C and e variants also contribute to Rh alloimmunization in patients with SCD [2], and thus *RH* genotyping is a valuable adjunct to serologic techniques to guide transfusion therapy and may provide an alternative approach to improve RBC matching. At our institution, prospective *RH* genotyping is performed for all patients with SCD, primarily to aid antibody evaluations and to provide antigen negative units to patients with partial Rh antigens on an individualized basis. Transfusion support can be tailored for patient-specific *RH* genotypes, and in the near future, high-throughput molecular approaches should improve RBC matching and allocation for patients requiring transfusion.

Acknowledgments

This study was supported in part by the Doris Duke Charitable Foundation Innovations in Clinical Research Award (S.T.C., C.M.W.) and the NICHD T32 Pediatric Pharmacoeconomics Training Program (J.J.W., Grant 5T32HD064567). We thank Mitchell J. Weiss for contributing the photo of the patient's serial urine samples, and for helpful discussion.

Sources of Support: The Doris Duke Charitable Foundation

References

1. Smith-Whitley K, Thompson AA. Indications and complications of transfusions in sickle cell disease. *Pediatr Blood Cancer*. 2012; 59(2):358–364. [PubMed: 22566388]
2. Chou ST, Jackson T, Vege S, et al. High prevalence of red blood alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. *Blood*. 2013; 122(6):1062–1071. [PubMed: 23723452]

3. O'Suoji C, Liem RI, Mack AK, et al. Alloimmunization in Sickle Cell Anemia in the Era of Extended Red Cell Typing. *Pediatr Blood Cancer*. 2013; 60(9):1487–1491. [PubMed: 23508932]
4. Miller ST, Kim HY, Weiner DL, et al. Red blood cell alloimmunization in sickle cell disease: prevalence in 2010. *Transfusion*. 2013; 53(4):704–709. [PubMed: 22804353]
5. Silvy M, Tournamille C, Babinet J, et al. Red blood cell immunization in sickle cell disease: evidence of a large responder group and a low rate of anti Rh linked to partial Rh phenotype. *Haematologica*. 2014
6. Win N. Hyperhemolysis syndrome in sickle cell disease. *Expert Rev Hematol*. 2009; 2(2):111–115. [PubMed: 21083443]
7. Lasalle-Williams M, Nuss R, Le T, et al. Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center. *Transfusion*. 2011; 51:1732–1739. [PubMed: 21332724]
8. Wagner FF, Ladewig B, Angert KS, et al. The DAU allele cluster of the RHD gene. *Blood*. 2002; 100(1):306–311. [PubMed: 12070041]
9. Talano JA, Hillery CA, Gottschall JL, et al. Delayed hemolytic transfusion reaction/hyperhemolysis syndrome in children with sickle cell disease. *Pediatrics*. 2003; 111(6 Pt 1):e661–665. [PubMed: 12777582]
10. Roubinet F, Apoil PA, Blancher A. Frequency of partial D phenotypes in the south western region of France. *Transfus Clin Biol*. 1996; 3(4):247–255. [PubMed: 8933677]
11. Reid ME, Hipsky CH, Hue-Roye K, et al. Genomic analyses of RH alleles to improve transfusion therapy in patients with sickle cell disease. *Blood Cells Mol Dis*. 2013
12. Gorst DW, Rawlinson VI, Merry AH, et al. Positive direct antiglobulin test in normal individuals. *Vox Sang*. 1980; 38(2):99–105. [PubMed: 6967653]
13. Zantek ND, Koepsell SA, Tharp DR Jr, et al. The direct antiglobulin test: a critical step in the evaluation of hemolysis. *Am J Hematol*. 2012; 87(7):707–709. [PubMed: 22566278]

Figure 1. Delayed hemolytic transfusion reaction due to anti-D in a D+ patient with sickle cell disease

A. Hemoglobin and reticulocyte count pre- (day 0) and post-red blood cell transfusion on days indicated. **B.** Patient's serum on day of presentation. **C.** Patient's urine on presentation and every 6 hours for the first 24 hours of admission (from left to right). **D.** Predicted red cell mass at baseline, immediately post-transfusion (day 0, D0), on day of admission (10 days post-transfusion, D10), and 16 days after transfusion (D16). Red cell mass was calculated as $70 \text{ mL/kg} \times \text{weight (kg)} \times \text{hematocrit}$.

Table 1
Blood Bank Evaluation

Days from transfusion	D typing	Antibody evaluation	DAT
Pre-transfusion	1+	Negative	Not done
Day 10	3+ mixed field	Anti-D 1+	Negative
Day 11	2+ mixed field	Anti-D 2+	Not done
Day 15	Weak+	Anti-D 4+	Negative

Mixed field D typing indicates a specimen containing red blood cells (RBCs) with strong D typing (transfused RBCs in this case) and with weak D typing (the patient's RBCs). The DAT is expected to be positive during a DHTR, but can be negative with substantial clearance of transfused RBCs. The interpretation and significance of a positive DAT must be correlated with the patient's clinical history and findings. Of note, a positive DAT is found in 1 in 1,000 to 14,000 healthy blood donors [12], and in 7 to 8% of hospitalized patients [13].