Severe delayed hemolytic transfusion reaction due to anti-Fy3 in a patient with sickle cell disease undergoing red cell exchange prior to hematopoietic progenitor cell collection for gene therapy

To our knowledge, most patients with sickle cell disease (SCD) who have undergone hematopoietic progenitor cell (HPC) collection for gene therapy have been conditioned with a series of monthly simple or exchange transfusions. The rationale for this practice is to improve safety and decrease bone marrow inflammation, potentially leading to higher $CD34^+$ mobilization and decreased risk of adverse events during HPC collection. Whether transfusion actually improves $CD34^+$ mobilization is not clear however, and we previously showed that patients can be safely and effectively mobilized without prior transfusion.[1] The risk of delayed hemolytic transfusion reactions (DHTR) is well known and notably appears to be higher in patients who are episodically, rather than chronically, transfused.[2] Here we report the case of a 34-year-old male with SCD (genotype SS) who had a severe DHTR after a single red cell exchange (RCE) performed prior to plerixafor mobilization and autologous HPC collection for a study protocol.

The patient has a history of multiple SCD-related complications, including aplastic crisis, pneumonia, recurrent acute chest syndrome, recurrent priapism, and silent cerebral infarcts. He has never needed regular transfusions but had occasional transfusions for acute chest syndrome and pain crises. His symptoms were controlled on hydroxyurea (HU), achieving a hemoglobin (Hb) F of ~20%. As per our protocol, HU was discontinued for 3 weeks prior to mobilization; 1 week prior to mobilization his HbF of 19% remained at his HU-treated baseline.

The patient is group A RhD-positive and K-negative. His primary medical care facility for the last 10 years provided red blood cells (RBC) for transfusion matched for CEK and antigens to which he had made antibodies, but he had received transfusions at other hospitals with unknown antigen matching standards. His last transfusion was 4 years prior to this RCE. At the time of RCE, his direct antiglobulin test (DAT) was negative and his antibody (Ab) screen showed anti-E, anti-Fy^a, and anti-Le^a; the anti-Fy^a and anti-Le^a had been detected 5 and 6 years prior, respectively. Although not currently detected, he had a history of anti-Jk^a from his primary medical facility but the anti-Jk^a was not detected when samples were tested by the reference laboratory. RBC genotyping (see Table 1) agreed with his RBC Ab history except his RBC were predicted to be Jk(a+) by human erythrocyte antigen (HEA) test. Subsequent sequencing confirmed the predicted *JK*A/B* genotype and a mutation that does not encode an amino acid change; RNA testing showed the presence of JK*A and JK*B transcripts, consistent with the patient's RBC expressing Jka and Jkb antigens. He was also found to have a common mutation in the GATA-1 binding motif of the erythroid *FY*B* promoter, which silences Fyb expression on erythrocytes but not on other cells of his body.[3,4]

Despite his red cell Ab history, he had no history of symptomatic hemolytic transfusion reactions, hyperhemolysis, or warm autoimmune hemolytic anemia. As per study protocol, on the day prior to stem cell mobilization and collection, the patient underwent RCE without adverse events with nine crossmatch-compatible red cell units that were group A, D+, E–, K–, and Fy(a–). Although anti-Le^a was microscopically positive at polyethylene glycol (PEG) IgG, anti-Lea is not routinely matched for at the study institution nor had been matched for with transfusions at his primary medical facility. After RCE, his Hb rose from 9.3 to 11.2 g/dL, and his HbS% decreased from 78% to 22% (HbA% of 72%). The following day, the patient was given a single 240 µg/kg dose of plerixafor for HPC mobilization and started leukapheresis about 3 hours afterward. He had no adverse events and was discharged home after his leukapheresis.

Five days after RCE, the patient developed throbbing low back pain, subjective fevers, dark urine, and scleral icterus, symptoms differing from his typical vaso-occlusive crises usually manifesting as right-sided flank/upper back discomfort and priapism. He contacted the on-call research study provider the day after symptom onset and then his sickle cell physician 2 days after symptom onset, who instructed the patient to go to the Emergency Department (ED) for work-up. When he presented to the ED closest to him on the third day after symptom onset, he was found to have a fever of 38.8°C, tachycardia to 131 bpm, blood pressure of 133/78 mm Hg, and an oxygen saturation of 93% by pulse oximeter. His labs were significant for a hemoglobin of 6.1 g/dL, hematocrit (Hct) of 17.0%, reticulocyte count of 18%, total bilirubin of 4.7 mg/dL with indirect bilirubin of 3.8 mg/dL (baseline total bilirubin of 2.3 mg/dL, baseline indirect bilirubin of 1.5), lactate dehydrogenase of 875 U/L (baseline lactate dehydrogenase 408 U/L), haptoglobin of <20 mg/dL, and hemoglobin S% of 77%. His urinalysis was positive for bilirubin, blood, and protein. His Ab screen was positive, and his RBC reacted in the DAT (1+W) with anti-IgG and anti-C3. Ab investigation was performed with low ionic strength salt solution, papain, and PEG indirect antiglobulin tests (IAT), and the patient's serum demonstrated reactivity with all cells positive for Fy^a and Fy^b, even with papain treatment, which abolishes Fy^a and Fy^b expression on RBC but does not affect Fy3. These results pointed to a new alloantibody, anti-Fy3. The patient was diagnosed with a DHTR. His hospital course was complicated by worsening hypoxemia with fevers, and he was found to have segmental pulmonary emboli in the bilateral lower lobes and possible pneumonia. He was treated with supplemental oxygen, enoxaparin, antibiotics, fluids and opioids for pain control; further transfusion was avoided given hemodynamic stability and fear of inducing hyperhemolysis.

In addition to the newly detected antibody that was directed against the Fy3-antigen of the Duffy blood group system,[5] the previously detected anti-E, anti-Fy^a, and anti-Le^a were also present. Furthermore, the patient's plasma reacted weakly with all E–, Fy(a–b–) and Le(a–) panel cells by papain and PEG IAT. An acid eluate prepared from the patient's red cells reacted weakly with all cells tested by PEG IAT, consistent with the presence of a warm autoantibody. Lookback on the red cell units transfused during the exchange showed that 8 of the 9 units were Fy(a-b+); only 1 of the 9 units was Fy(a–b–). All units were Jk(a+); three units had Le^a typing available and were Le(a-). Therefore, if hemolysis was from the newly detected anti-Fy3, he would be expected to lose 7.2 grams/dL of Hb due to hemolysis of the transfused Fy(a–b+), resulting in a patient hemoglobin of 4 g/dL (see Figure 1). Indeed, his Hb nadired at 4.4 g/dL, suggesting hemolysis of Fy(a–b+) cells.

Table 1. Human erythrocyte antigen (HEA) phenotype by DNA analysis report.

Rh								Kell				Kidd		Duffy		MNS				
c	C	e	E	V	VS	K	k	Kpa	Kpb	Jsa	Jsb	Jka	Jkb	Fya	Fyb	M	N	S	s	U
+*	+	+	0	+	+	0	+	0	+	0	+	+	+	0	(0)#	+	+	+	+	+

+* = c+ (partial). (0)# = GATA silencing mutation for Fy^b.

He was discharged home 14 days after his initial ED presentation on anticoagulation. Approximately 6 weeks post-discharge, his Hb and Hct had improved to 8.4 g/dL and 22.4%, respectively, and he was restarted on hydroxyurea. He has not required further transfusion since his DHTR event. A follow-up Ab screen at his primary medical care facility 67 days after his RCE was positive and demonstrated anti-E, anti-Fya, and anti-Lea. The anti-Fy3 was no longer demonstrable.

There are only five prior case reports in the literature describing acute and delayed hemolytic transfusion reactions due to anti-Fy3,[6-10] an antigen in the Duffy blood group system. This system is comprised of five antigens, but antibodies against only three antigens, Fy^a, Fy^b and Fy3, are known to cause hemolytic transfusion reactions. Fy^a and Fy^b antigens differ by a single point mutation, while Fy3 refers to a common epitope on the FY protein separate from the Fy^a/Fy^b polymorphism (Figure 2). The four major phenotypes are Fy(a+b+), Fy(a+b–), Fy(a–b+), or Fy(a–b–). Approximately 68% of Africans and people of African descent have lost expression of the FY protein on RBC and phenotype as Fy(a–b–); FY loss protects against malarial invasion because FY is a receptor for the malarial parasites *P. vivax* and *P. knowlesi*.[3,11] The absence of FY protein on the RBC is primarily due to a point mutation in the GATA promotor region on a FYB allele such that the erythroid transcription factor GATA-1 fails to bind;[12] thus, RBC lack expression of Fy^b.[3] Patients with the promoter mutation are not at risk for anti-Fyb, despite serologically typing as Fy(b-), because they have a structurally normal FY*B allele and Fy^b is expressed on other tissues. These patients remain at risk for anti-Fy3, which can be clinically significant, and patients with an existing anti-Fy^a are at increased risk.[9-13] The reasons for this have long been studied but are yet to be determined. We speculate that DHTR due to anti-Fy3 may be rare for several reasons: Fy3 may be of low immunogenicity; anti-Fy3 has been under-recognized as a cause of DHTR; and C–E– RBC are likely to also be Fy(a–b–). We speculate that patients with an existing anti-Fy^a may develop anti-Fy3 if their Fy3 tissue promoter is defective and thus also become immunized to Fy3 antigen at the time of stimulation to Fy^a.

This case illustrates several important points. First, this patient's symptoms were initially not recognized as a potential DHTR. As gene therapy treatments for SCD become more common, transplant providers with less experience in treating patients with SCD should be educated regarding SCD-specific complications that may be encountered during treatment. Secondly, we suggest extended antigen matching for additional clinically significant antigens for episodically transfused immune red cell responders, i.e, patients with multiple alloantibodies. Importantly, in those who have made anti-Fy^a and are Fy(a–b–) due to the GATA mutation, Fy(a–b–) units should be considered, if possible, especially with high volume RCE. Fy(a–b–) units are primarily found in blood donors of African ancestry; matching may be difficult in patients with multiple alloantibodies. Thirdly, this case illustrates the importance of optimal transfusion management of patients with SCD, which includes red cell genotyping, performing antibody screens approximately 1 month post-transfusion to detect newly formed alloantibodies that may evanesce, and establishing a nationwide patient database of prior red cell antigen profiles and red cell antibody history that is available across hospital systems. The time frame of the appearance of anti-Fy3 is consistent with an anamnestic response to an antibody that, to our knowledge, was not previously detected. Finally, although it is standard practice to perform simple

$$\text{Patient Hb from } Fy3^+ \text{ units} = (\%\ Fy3^+ \text{ units}) \times (\%\ \text{HbA}) \times (\text{total Hb post exchange}) = 0.89 \times 0.72 \times 11.2\ \frac{g}{dL} = 7.2\frac{g}{dL}$$

$$\text{Predicted patient Hb post hemolysis} = (\text{Hb post exchange}) - (\text{Hb from } Fy3^+ \text{ units}) = 11.2\frac{g}{dL} - 7.2\frac{g}{dL} = 4\frac{g}{dL}$$

Figure 1. Estimations of amounts of Fy3+ hemoglobin post exchange and of total hemoglobin post Fy3+-specific hemolysis. In order to estimate the amount of Fy3+ blood transfused in grams/dL (g/dL), the percentage of Fy3+ units (8 of 9 units) was multiplied by the percentage of hemoglobin A (HbA) post-exchange and multipled by the total hemoglobin (Hb) post-exchange in g/dL. In order to estimate the total Hb after hemolysis of the Fy3+ units, the amount of Hb from the Fy3+ units was subtracted from the total Hb post-exchange.

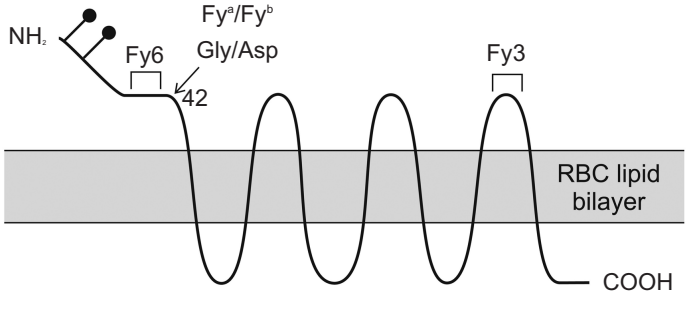

Figure 2. Diagram representing the predicted 7-transmembrane domain structure of the Duffy glycoprotein. Indicated are the amino and carboxy terminals and location of the Fy^a/Fy^b polymorphism at amino acid 42. Also indicated, on the third extracellular loop, is the predicted location of the sequences necessary for the binding of monoclonal anti-Fy3. The actual sequence required for binding of human polyclonal anti-Fy3 is not known. Also shown is the predicted region for binding of monoclonal anti-Fy6. N-glycosylation sites are indicated by ⁂.

or exchange transfusions prior to stem cell mobilization with plerixafor in patients with SCD, studies are needed to determine whether treatment modalities other than transfusion, such as hydroxyurea[1] or other newly approved drugs, can be effective alternatives prior to plerixafor mobilization. In addition to the risk of serious DHTR, transfusion does not necessarily lead to good mobilization[14] and also may not be an option for highly alloimmunized or Jehovah's Witness patients.

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doi:10.3324/haematol.2020.253229

Disclosures: no conflicts of interest to disclose.

Contributions: EFS and PAS wrote the manuscript; STA, DLW, DLD, FB, and PAS cared for the patient, CLF and CMW performed immunohematology studies, and all authors edited or reviewed the manuscript.

Acknowledgments: the authors would like to thank our patient for his study participation; the Doris Duke Charitable Foundation for a 2011 Innovation in Clinical Research Award for trial support (to PAS and MS); and Sanofi-Genzyme for provision of plerixafor.

Funding: this work was also supported by the Memorial Sloan Kettering Core Grant (P30 CA008748) funded by the National Cancer Institute.

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