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Utility of hemoglobin electrophoresis to distinguish between severe delayed hemolytic transfusion reaction versus hyperhemolysis syndrome

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ABSTRACT

Delayed hemolytic transfusion reaction (DHTR) and hyperhemolysis syndrome (HHS) are both complications of red blood cell transfusions in patients with sickle cell disease.Clinically, both present with hemolysis and can be difficult to differentiate. Hemoglobin electrophoresis may aid in the diagnosis. Herein we describe a case in which a patient with hemoglobin SC disease presented with features of severe hemolysis several days after initiation of red blood cell exchange. Increase in reticulocyte count and complete absence of hemoglobin A on electrophoresis during this event supported the diagnosis of severe DHTR, indicating a rapid and selective destruction of the transfused red blood cells. Ability to interpret the hemoglobin electrophoresis can help clinicians distinguish between these two severe transfusion complications in patients living with sickle cell disease. It is important to identify the presence or absence of concomitant HHS, as patients with HHS tend to have a worse prognosis and there is a higher rate of recurrence of HHS with subsequent transfusions. Accurate diagnosis can lead to prompt management and decrease morbidity and mortality.

1. Introduction

Patients with sickle cell disease (SCD) commonly receive red blood cell (RBC) transfusions for several indications, throughout their lifetime. Severe delayed hemolytic transfusion reaction (DHTR) is a potential complication of transfusion and can develop into hyperhemolysis syndrome (HHS). Per ASH guidelines, DHTR is defined as a significant drop in hemoglobin within 21 days post-transfusion along with formation of a new red cell alloantibody, hemoglobinuria, increased hemoglobin S, decrease in hemoglobin A, rise in lactate dehydrogenase (LDH) from baseline in the absence of alternative causes [1]. Alternatively, according to the CDC National Healthcare Safety Network Hemovigilance criteria, DHTR is defined as a positive DAT for antibodies developed between 24 h and 28 days after transfusion, along with a positive eluate or a newly-identified antibody, and an inadequate rise of post-transfusion hemoglobin or a rapid drop in hemoglobin to pre-transfusion levels, or unexplained spherocytes on peripheral smear. Subtle differences in the definition of DHTR may contribute to variability in the diagnosis of DHTR [2].

Reported incidence of DHTR varies significantly. A prospective observational study from a single institution reported that as many as 4.2% of episodic transfusions in patients with SCD result in DHTR [3]. The pathophysiology of DHTR is due to a recipient's production of alloantibodies against a donor's RBC antigens following a transfusion with re-exposure to the same RBC antigen triggering an anamnestic antibody response resulting in complement activation and hemolysis of allogeneic RBCs [4].

Hyperhemolysis syndrome (HHS) is a severe form of DHTR that is accompanied by bystander hemolysis of the patient's own red cells. HHS presents with fever, jaundice, signs and symptoms of hemolytic anemia, and severe pain usually out of proportion to patient's known vaso-occlusive pain. HHS may present with a negative or a positive direct antiglobulin test (DAT) and with or without detection of new alloantibodies. The pathophysiology of HHS is characterized by destruction of both allogeneic and autologous RBCs, with hemoglobin levels falling below the level prior to the transfusion [4]. The pathogenesis is unclear, but several theories have been proposed, including macrophage activation, complement mediated-hemolysis, and antibody-dependent hemolysis. Reticulocytopenia is also a well-documented manifestation of HHS, either due to myelosuppression, peripheral destruction by macrophages, or bystander hemolysis. Bystander hemolysis occurs when complement is activated against both the transfused red cells and the

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patient's own red cells. HHS is associated with significant morbidity and mortality with a high risk of recurrence in the setting of further transfusion [5].

2. Case presentation

A 54-year-old man with HbSC disease complicated by recurrent cerebrovascular accidents (CVA) presented for a second opinion at our clinic. His sickle cell history included a remote episode of acute chest syndrome and subsequent multi-organ dysfunction requiring red blood cell exchange transfusion. Given his history of recurrent CVAs, the patient was recommended to undergo monthly red blood cell exchange for secondary stroke prevention [6]. After obtaining his antibody information from the prior institution where he was last transfused, the patient underwent red blood cell exchange with six (1850 mls) crossmatch-compatible packed red cell units. Pre-exchange transfusion, the patient had a history of anti-K, anti-Jk^b and anti-Fy^a. Transfused red cells were HbS-negative and phenotypically matched for Rh, Kell, Kidd and Duffy antigens. Patient exhibited a Fy^b GATA promoter silencing mutation, and therefore Fy^b-antigen negative units were not selected. Baseline hemoglobin prior to the exchange transfusion was 8.1 g/dL. Hemoglobin immediately after exchange transfusion was 10.8 g/dL. Hemoglobin electrophoresis immediately following exchange transfusion demonstrated HbA 53.7%, HbF 2.8%, HbA_2 2.2%, HbS 14.6%, HbC 12.8%, and 13.9% other Hb. Other fraction was suggestive of an alpha chain variant; quantification included the peak for alpha chain variant hemoglobin and the SC/alpha chain hybrid hemoglobin.

Eight days later, the patient presented to an outside hospital with a few days of severe left thigh, left hip pain, as well as dark brown urine. He reported his pain as significantly higher than what he typically experienced with a vaso-occlusive pain crisis. Upon arrival, he was initially afebrile, normotensive and saturating well on room air. Labs showed a hemoglobin of 9.5 g/dL, white blood cell count of 13.8×10^9 /L, reticulocyte count of 4.3% (increased from patient's historic reticulocyte count of 2.06%, absolute 0.071 M/mcl), total bilirubin of 5.6 mg/dL, direct bilirubin 0.9 mg/dL, LDH 1504 U/L, undetectable haptoglobin, elevated creatinine 1.67 mg/dL (baseline 1.1), aspartate aminotransferase (AST) 81 U/L, and alanine transaminase (ALT) 28 U/L. He became febrile that evening with a temperature of 39.5 C and was started on ceftriaxone.

On hospital day (HD)+ 1, labs showed a significant hemoglobin drop to 6.6 g/dL, and a worsening transaminitis with AST 436 U/L, ALT 115 U/L, and total bilirubin increasing to 6.3 mg/dL. Blood bank testing revealed numerous new antibodies, including anti-Le^a, Anti-N, cold auto antibody, and anti-Fy^3. On HD + 1, DAT was positive with anti-IgG and the eluate studies performed at a reference laboratory showed that anti-Fy^3 was found to be coating the red blood cells. The patient was diagnosed with a severe hemolytic reaction and initiated on intravenous immunoglobulin (IVIG) 400 mg/kg, prednisone 1 mg/kg, and epoetin alfa 20,000 units daily. Kidney function continued to worsen, and creatinine peaked at 3.1 mg/dL on HD+ 3. Hemoglobin continued to decrease reaching 3.6 g/dL on HD+ 4. He was transferred to a tertiary care center on HD+ 4.

Upon arrival to the tertiary care center (HD +4), patient's DAT was repeat positive with anti-IgG. Patient's hemoglobin continued to decline with a nadir of 2.9 g/dL on HD+ 5. LDH peaked at 1513 U/L and reticulocyte count increased to 6.6% on HD+ 6. Repeat hemoglobin electrophoresis on HD+ 8 revealed HbA 0%, HbF 6%, $HbA_2$1.6%, HbS 30.9%, and HbC 28.2%, and 33.3% other hemoglobin (Table 1). Retic count peaked at 20.55% (absolute count of 0.261 M/mcl) on HD+ 9. He completed a five-day course of IVIG, a 9-day course of prednisone 1 mg/kg, and epoetin alfa 20,000 units daily for 10 days. At discharge on HD+ 12, the hemoglobin had increased to 5.8 g/dL. Acute kidney injury was improving at discharge, with creatinine down to 1.91 mg/dL. Total bilirubin was 1.4 mg/dL, AST 50 U/L, and ALT 56 U/L at discharge. After discharge, patient received weekly CBCs. The patient returned to our outpatient clinic on HD + 45 and had a hemoglobin of 6.5 mg/dL.

Table 1
Table demonstrates hemoglobin electrophoresis results before, immediately following a red blood cell exchange transfusion (on hospital day −8) and after the patient presented to the hospital following a suspected transfusion reaction. All of the transfused hemoglobin A red blood cells were completely hemolyzed due to the hemolytic transfusion reaction.

	Immediately before RBC Exchange	Immediately after RBC Exchange	Hospital Day + 8
Total Hemoglobin	8.1 g/dL	10.8 g/dL	4.0 g/dL
Hemoglobin A	0%	53.7% (5.8 g/dL)**	0.0% (0 g/dL)**
Hemoglobin F	5.0%	2.8%	6.0%
Hemoglobin A2	1.8%	2.2%	1.6%
Hemoglobin S	31.7%	14.6% (1.58 g/dL)**	30.9% (1.24 g/dL)**
Hemoglobin C	28.0%	12.8% (1.38 g/dL)**	28.2% (1.13 g/dL)**
Hemoglobin Other*	33.5%	13.9%	33.3%

* Other: suggestive of an alpha chain variant. Other fraction includes the peak for alpha chain variant hemoglobin and the SC/alpha chain hybrid hemoglobin.
** Hb in g/dL = Fraction % x Total Hemoglobin level

3. Discussion

Herein, we describe a patient with HbSC disease who presented with severe hemolysis following a red blood cell exchange transfusion. He had severe anemia, reticulocytosis, a positive DAT, new alloantibodies, and evidence of hemolysis. Given the precipitous drop in the patient's hemoglobin, and a drop in Hb A, the patient was diagnosed with severe DHTR [1,7]. Severity of hemolysis also raised concern for concomitant HHS. The patient had reticulocytosis, while greater than 80% of HHS cases are accompanied by reticulocytopenia [5]. In addition, a hemoglobin electrophoresis demonstrated complete elimination of HbA red cells with the pattern reverting back to the patient's pre-exchange transfusion baseline profile (Table 1). In HHS there is typically an associated marked decrease in sickle red cells, consistent with bystander hemolysis of patients own red cells [8]. Our patient demonstrated a near elimination of HbA red cells (drop from 7 g/dL to 0 g/dL), while the Hb S+C red cells showed a mild decrease (drop from 2.96 g/dL to 2.37 g/dL) (Table 1). The marked drop in overall hemoglobin was attributed to the development of a clinically significant anti-Fy3 antibody, which caused destruction of the multiply transfused red cells. Fy3 is a high prevalence antigen in the Caucasian population, but has a prevalence of 32% in Black population [9]. While the Fy3 antigen status of transfused red cells remain unknown, per communication with the regional blood center, one of the six units transfused were from a donor who was known to be positive for the Fyb antigen; the remaining five units were from donors who remain untested for the Fyb antigen. Hence, none of the six donors were known to have a FY_{null} [FY: − 3; Fy (a-b-)] phenotype.

A diagnosis of severe DHTR without HHS was favored given the formation of a new antibody, presence of reticulocytosis, and the hemoglobin electrophoresis pattern. It is important to identify if there is a concomitant HHS, as HHS is associated with worse prognosis and a high risk of recurrence with subsequent transfusions. One retrospective case-control analysis demonstrated that of the 41 patients with 54 episodes of HHS, 13% of the episodes were fatal and HHS recurred in 52.6% of patients [5].

There is currently no validated treatment for management of DHTR with or without HHS, as no randomized-control studies have been performed [10]. However, the main tenants of DHTR management include supportive care, optimization of erythropoiesis, minimizing future blood transfusions, and consideration of immunomodulatory therapies [4]. Supportive care includes intravenous fluids, oxygenation, folic acid

supplementation, and analgesia with opioids. Erythropoiesis is stimulated by administering high doses of erythropoietin. Immunomodulatory therapies include IVIG and high dose corticosteroids. Steroid use should be weighed against the risk of worsening a vaso-occlusive crisis. Eculizumab, an anti-C5 antibody can be used in HHS to inhibit terminal complement pathway activation. Tocilizumab, a monoclonal antibody against interleukin (IL)− 6, has been tried in refractory cases of HHS [11]. Avoidance of future transfusions in patients with HHS is recommended with careful consultation with transfusion medicine experts if blood is ever emergently required. In patients that are at a high risk of DHTR given prior history of alloimmunization, transfusions should be avoided if possible. If transfusion becomes necessary, these patients should be transfused with extended phenotypic matched units with increased vigilance for signs and symptoms of hemolysis.

4. Conclusion

Hemoglobin electrophoresis along with reticulocyte count may aid in determining the presence or absence of concomitant HHS in patients presenting with severe DHTR. Making the correct diagnosis has implications in the prognosis of the patient, as well as for potential future transfusion reactions.

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Robert Lukin: Writing – original draft. **Jennie Y Law:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Parvez M. Lokhandwala:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

None.

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