Hyperhemolysis syndrome in a patient without a hemoglobinopathy, unresponsive to treatment with eculizumab

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BACKGROUND: Hyperhemolysis is a serious transfusion reaction, most often described in patients with hemoglobinopathies. Hyperhemolysis is characterized by the destruction of host red blood cells (RBCs), in addition to donor RBCs, via an unknown mechanism.

STUDY DESIGN AND METHODS: We present the case of a 58-year-old woman with treated human immunodeficiency virus and a normal hemoglobin (Hb) electrophoresis who developed hyperhemolysis in the setting of a delayed hemolytic transfusion reaction (DHTR).

RESULTS: The patient was ABO group B and had a previously identified anti-Fyb alloantibody. After transfusion of Fyb– RBCs, she developed a DHTR and was found to have anti-E, anti-Cw, anti-s, and an additional antibody to an unrecognized high-frequency RBC alloantigen. Subsequent transfusion of ABO-compatible RBCs that were negative for Fyb, E, Cw, and s antigens resulted in immediate intravascular hemolysis. In the absence of bleeding, her hematocrit (Hct) decreased to 10.2%. An extensive serologic evaluation failed to identify the specificity of the high-frequency antibody. Severe hemolytic reactions also occurred despite pretransfusion conditioning with eculizumab. The Hct and clinical symptoms slowly improved after the cessation of transfusions and treatment with erythropoietin and steroids. This case demonstrates several noteworthy features including hyperhemolysis in a patient without a Hb disorder, the development of an antibody to an unknown RBC antigen, and the failure of eculizumab to prevent intravascular hemolysis after transfusion.

CONCLUSION: Hyperhemolysis is not restricted to patients with hemoglobinopathies. Whether eculizumab offers any benefit in the hyperhemolysis syndrome or in the prevention of intravascular hemolysis due to RBC alloantibodies remains uncertain.

Hyperhemolysis is a rare syndrome of life-threatening hemolysis that can occur in the context of a delayed hemolytic transfusion reaction (DHTR). Patients with hyperhemolysis experience persistent red blood cell (RBC) hemolysis in which both the donor RBCs and the patient’s own RBCs are destroyed.1 As a result, hemoglobin (Hb) levels can fall to life-threatening levels. Subsequent transfusions, even if serologically compatible, undergo rapid destruction. Unlike autoimmune hemolytic anemia, the direct antiglobulin test (DAT) in patients with the hyperhemolysis syndrome may be negative or, if positive, may reveal antibodies in the eluate directed against antigens no longer found on circulating cells of the sample taken from the patient. Although the syndrome has most often been described in heavily transfused patients with hemoglobinopathies such as sickle cell disease,2,3 it has also been occasionally reported among individuals with other conditions.4,5

We present the case of a woman with chronic hepatitis C and human immunodeficiency virus (HIV) infections who developed hyperhemolysis after a DHTR in the presence of four alloantibodies (anti-E, anti-Fyb, anti-Cw, anti-s) plus a fourth alloantibody directed against an unidentified high-frequency antigen. The patient demonstrated hemolysis of her own RBCs despite a negative DAT. Transfusions of E–, Cw–, Fyb–, and s– RBCs resulted in

ABBREVIATIONS: CT = computerized tomography; DHTR = delayed hemolytic transfusion reaction; HLH = hematophagocytic lymphohistiocytosis.

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Received for publication June 29, 2014; revision received August 11, 2014, and accepted August 11, 2014.
doi: 10.1111/trf.12876
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immediate intravascular lysis. Lysis of transfused blood was not blocked despite treatment with high-dose eculizumab. She developed life-threatening anemia and was treated with supplemental oxygen, corticosteroids, and erythropoietin (EPO) while further transfusions were withheld. Her hemolysis abated and she ultimately recovered to her baseline hematocrit (Hct). She was readmitted approximately 12 weeks later with acute cholecystitis and found to have pigment gallstones consistent with her history of hemolysis.

CASE REPORT

A 58-year-old woman with a history of HIV and hepatitis C infections, and chronic obstructive pulmonary disease, was transferred to our hospital for worsening anemia. Her baseline Hb and Hct were approximately 10 g/dL and 30%, respectively. Over the preceding 2 years, she had been treated for Lyme disease and for five episodes of pneumonia. Three weeks before her transfer she was admitted to a hospital for dyspnea and cough and was diagnosed with methicillin-resistant Staphylococcus aureus pneumonia. She was treated with intravenous (IV) vancomycin, followed by doxycycline, which resulted in a rash. The antibiotics were switched to levofloxacin and trimethoprim/sulfamethoxazole, and she was discharged. She became progressively light-headed and had dyspnea on exertion and was readmitted. Repeat laboratory examination now revealed a Hct of 17.9%, a white blood cell (WBC) count of $2.9 \times 10^9/L$, and a platelet (PLT) count of $128 \times 10^9/L$.

She underwent an extensive laboratory evaluation. Her reticulocyte count was 0.25%. Serologies for Epstein-Barr virus, cytomegalovirus, and parvovirus were consistent with past infection. Testing for glucose-6-phosphate dehydrogenase deficiency was normal. A marrow biopsy revealed a hypocellular marrow with erythroid hypoplasia, left-shifted myeloid elements, and megakaryocytic hyperplasia. There was no infiltrative process and no granulomas, and stains for acid-fast bacilli and Pneumocystis jiroveci were negative. Flow cytometry of the marrow cells did not reveal a monoclonal cell population. An abdominal ultrasound demonstrated mild splenomegaly (14.8 cm).

To address her anemia, she was transfused with 3 units of RBCs on Hospital Day 1 (Fig. 1). On Day 2, her Hct had improved to 25.9%. However, on Days 3 and 4, her Hct declined to 23.9 and 23.1%, respectively. On Days 5 through 12, the Hct continued to decline despite repeated transfusions of cross-match–compatible blood. On Days 11 through 14, she received supplemental EPO. On Day 14, she was given 125 mg of IV methylprednisone and was transferred to our hospital.

At our institution, she confirmed her fatigue, malaise, exercise intolerance, and mild substernal chest pressure with any exertion. She noted that her urine and stool were “rusty colored.” She described a 20-pound weight loss over the past 3 months, stating that her appetite had been poor. She had no fevers or chills, and no nausea or vomiting, but reported diarrhea 4 to 5 days before admission, which had since resolved. Her only travel had been to the Dominican Republic, Mexico, and Florida. She was retired from her job at a local drug treatment center.

Physical examination revealed tachycardia but otherwise normal vital signs. She was noted to have coarse breath sounds diffusely throughout both lungs. She had no rashes or adenopathy. Initial laboratory studies showed a Hct of 12.1%, WBC count of $3.0 \times 10^9/L$ (78% polymorphonuclear leukocytes, 17.5% lymphocytes, and 3.4% monocytes), and a PLT count of $95 \times 10^9/L$. Her ferritin was 1800 ng/mL, lactate dehydrogenase (LDH) of 1300 U/L (normal 110-210 U/L), and haptoglobin less than 6 mg/dL. Her serum triglycerides were 425 mg/dL. The electrocardiogram showed no evidence of ischemia, and her serum troponin was not elevated. A computerized tomography (CT) scan of the chest showed multiple bilateral nodules in the right upper, middle, and bilateral lower lobes; ground glass opacities prominent in the right lower lobe; and bronchiectasis. A CT scan of the abdomen and pelvis revealed splenomegaly (17.4 cm) but no evidence of adenopathy.

The patient was blood type group B, D+. Her history of prior transfusions included 4 units of RBCs in 2005 and 2 units of RBCs in 2008. She had a previously identified anti-Fyb alloantibody. Four days before her hospital transfer, and having received 4 units of RBCs, her DAT was found to be 2+ (IgG), and an eluate prepared from her RBCs and tested by the American Red Cross regional reference laboratory demonstrated an anti-s as well as a panagglutinin. Further evaluation by the American Red Cross National reference laboratory demonstrated anti-Fy$a^b$, anti-s, anti-E, anti-C$^c$, and an additional unidentified alloantibody.
At the time of transfer to Massachusetts General Hospital, the DAT was now weakly positive for IgG and the eluate was nonreactive consistent with serologic completion of a DHTTR. Multiple subsequent DATs were macroscopically nonreactive for the presence of either IgG or complement. After transfer to our hospital, an extensive evaluation of the patient’s antibodies was begun in collaboration with the American Red Cross. Her plasma was consistently incompatible with E− C− Fy(b−) s− cells. The hospital laboratory found that the patient’s RBCs tested positive for multiple high-frequency antigens and her plasma was reactive with target cells lacking high-frequency antigens.

Further evaluation by the American Red Cross National Reference Laboratory for Blood Group Serology focused on the alloantibody to a high-prevalence antigen. The unidentified antibody was reactive at the antiglobulin test phase using 22% albumin enhancement, reacted to a dilution of 128 with E− C− Fy(b−) s− RBCs, and the reactivity was weakened or removed when tested with reagent RBCs treated with dithiothreitol (DTT; American National Red Cross, Rockville, MD). All alloantibodies to common RBC antigens with the exception of anti-K were excluded using DTT-treated reagent RBCs. Examples of the following rare RBCs were reactive when tested with the patient’s serum: Kn(a−), Yk(a−), Sl(a−), Cs(a−), Rg−, Yt(a−), Liu(a−), Ge−2, IW(a−), MER2−, Jo(a−), TC(a−), U−, Vel−, PP−, P−, I−, H−, K−, K−11, K−12, K−27, Ka, M*M*, and cord cells. The antibody to a high-prevalence antigen was not removed by adsorption with rabbit erythrocyte stroma (RES; Immucor, Norcross, GA) or allogeneic RBCs. The patient’s plasma on E− Cw− K− N− s− Do(a−) showed reactivity at a dilution of 128 with E− C− Fy(b−) s− RBCs, and the reactivity was weakened or removed when tested with reagent RBCs treated with dithiothreitol.

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Investigational testing using a panel of lectins suggested the RBCs were not polyagglutinatable. Anti-s was recovered in an acid eluate (Gamma ELU-KIT II, Immucor) prepared from the initial sample but was not detected in an eluate prepared from a sample collected 5 days later. Samples from three siblings were submitted and were not compatible with the patient’s known alloantibodies in particular anti-Fyb or anti-s.

Subsequent testing and clinical course

DAT performed on a sample collected 2 weeks after the initial workup was still weakly reactive (+mMF) with anti-IgG. These cells were treated with chloroquine diphosphate (Sigma-Aldrich, St Louis, MO) to a negative DAT. A plasma sample collected 2 months after the initial investigation was nonreactive when tested with the chloroquine diphosphate–treated patient’s RBCs.

The sample collected 2 months later was reactive to a dilution of 64 with phenotypically similar donor RBCs. One of 22 E− C− K− N− s− Do(a−) donor samples was nonreactive with this sample when tested at the albumin-IgG-AGT phase. Investigational typing of the donor RBCs found it to be weakly positive for Yt and negative for K14 and Yk using a single source of unlicensed antisera. No additional anti-K14 or K−14 reagent cells were available to confirm the specificity. Two samples were sent to the International Blood Group Reference Laboratory in Bristol, UK. The initial sample was reactive with two examples of K0 RBCs. A subsequent sample collected 2 months later was reactive with one example each of U−, En(a−), and Ge−2,3,4 RBCs that were antigen negative for the known common antibodies. All 19 exons of the KEL gene were amplified by the polymerase chain reaction and directly sequenced. No mutations were found in the KEL gene.

All RBC units cross-matched for the patient were macroscopically (1+ to 2+) incompatible. The patient received several initial transfusions that failed to increase her Hct (Fig. 1) and that were accompanied by grossly visible hemoglobinuria. She remained symptomatic with fatigue and dyspnea on minimal exertion. In the absence of bleeding, the decreasing Hct suggested continued destruction of autologous cells. Because the immediate hemolysis of transfused cells suggested a complement-mediated process, she was given 600 mg of eculizumab IV during the 6 hours after the transfusions. Because the patient was not bleeding, we interpreted the failure of the Hct to increase within the first 6 hours of the transfusion to indicate ongoing destruction of transfused cells.

Because the clinical picture was consistent with the syndrome of hyperhemolysis, further transfusions were withheld and the patient was treated with supplemental...
oxygen by nasal cannula, corticosteroids, and supplemental EPO. All antibiotics were discontinued out of concern for marrow suppression or drug-induced hemolysis. Blood sampling for laboratory testing was minimized. Her Hct decreased to a nadir of 10.2%, but she remained hemodynamically stable with a pulse of 100 to 120. Her fatigue and dyspnea slowly improved, and she was discharged with a Hct of 21.9% after 33 days of hospitalization.

On Day 37, the patient returned to clinic without symptoms. Her Hct had increased to 28.1%. Her LDH and triglycerides were within normal limits. Her prednisone was decreased to 30 mg a day orally and then tapered over the next 3 weeks. She continued to receive supplemental EPO and by Day 51, her Hct was 41.3%. A repeat blood smear demonstrated rare tear drop RBCs, but was otherwise normal. The patient’s DAT remained negative and her plasma continued to demonstrate anti-Fy², anti-s, and anti-E plus a fourth alloantibody directed against a high-frequency antigen found on all target cells.

Because no compatible donor units had been identified, she remained on supplemental EPO to facilitate a course of three planned monthly autologous blood donations, so that her RBCs could be frozen for future emergency use. During scheduled follow-up in hematology clinic before her third autologous blood donation, she described a 1-day history of vague abdominal pain that was subsequently localizing to her right upper quadrant and epigastric regions. In clinic, her pain worsened and was accompanied by nausea and vomiting. An abdominal CT scan showed gallstones and gallbladder distension, terminal ileitis, and typhlitis consistent with acute cholecystitis. She was treated with cefoxitin before laparoscopic cholecystectomy. On pathologic inspection, her gallstones were noted to be pigment stones (Fig. 2). She had an uneventful postoperative course and was discharged with a Hct of 29%. One month later, a Hb electrophoresis demonstrated HbAA without evidence for HbS, C, or any variant Hb.

**DISCUSSION**

Hyperhemolysis syndrome is a rare but potentially catastrophic condition characterized by hemolysis of both transfused and autologous RBCs. Our case demonstrates a number of notable features of this rare syndrome: 1) It adds to the growing body of literature demonstrating that hyperhemolysis is not restricted to patients with hemoglobinopathies; 2) it occurred in the context of a DHTR that involved an alloantibody to an unknown high-frequency antigen that could not be resolved despite extensive reference laboratory investigation; and 3) it demonstrates the failure of eculizumab therapy to block acute intravascular lysis occurring at the time of transfusion.

Fig. 2. Gross appearance of pigmented gallstones removed on Day 126.

Our patient’s hyperhemolysis occurred in the setting of a DHTR that included an alloantibody to an unidentified high-frequency antigen. Hyperhemolysis triggered by a DHTR has previously been described in a patient without any hemoglobinopathy.¹ Despite intensive serologic evaluation by regional, national, and international reference laboratories, the specificity of the alloantibody could not be resolved. The antibody was incompatible with all donor target cells tested except the patient’s own cells. Genotyping of the patient failed to identify a candidate high-frequency antigen that was absent in the patient. The antibody appears to have resulted in immediate intravascular hemolysis, which was not blocked by treatment with eculizumab. Although our patient had a low reticulocyte response, the decreasing Hct, failure to respond to repeated RBC transfusions, markedly elevated LDH, absent haptoglobin, and gross hemoglobinuria all suggested a hemolytic process.

The syndrome of hyperhemolysis was first described in sickle cell patients² and later reported in patients with thalassemia.³,⁷ This case is distinctive as it occurred in a patient without a hemoglobinopathy. Patients with hyperhemolysis can display brisk hemolysis despite the transfusion of antigen-negative, cross-match–compatible RBCs. The hemolysis can begin within 1 to 7 days of transfusion, and in these cases the DAT is usually negative. However, the hemolysis can also be delayed (>7 days after transfusion), and in these cases the DAT is often positive.²,⁸ In our case, multiple DAT tests were nonreactive for complement. While we cannot be certain that the hemolysis was complement-mediated and thus subject to inhibition by eculizumab, the hemolysis after transfusion was immediate and accompanied by hemoglobinuria,
hemoglobinemia, and a decrease in Hct. We attributed the negative DAT result to immediate lysis of transfused cells.

The pathogenesis of hyperhemolysis is not well understood. The condition shares some features with cases of extended DHTR where the implicated alloantibody is recovered from the eluate even after antigen-positive cells have been cleared from the circulation.10,12 Our case, like another reported in a patient without a Hb disorder,8 shares this common feature of potentially being triggered by an alloantibody response. Hyperhemolysis syndrome also shares some features in common with the syndrome of hematophagocytic lymphohistiocytosis (HLH) including uncontrolled hemolysis, erythrophagocytosis, pancytopenia, and a highly elevated serum ferritin. In addition, our patient had elevated triglyceride levels that returned to normal after cessation of hemolysis, a feature also seen in HLH. As in HLH, inappropriate macrophage activation might explain the clinical response of hyperhemolysis to treatment with corticosteroids.11

The exact mechanism behind which this phenomenon occurs is unknown. Studies using high-performance liquid chromatography analysis in patients with hemoglobinuria after transfusion demonstrate both donor and recipient Hb in the urine, supporting the idea that there is a dual hemolytic response against both transfused and autologous RBCs.12 One proposed mechanism is that there is immune-mediated bystander hemolysis resulting from a defect in complement regulation; RBCs, particularly sickle cells, become “innocent bystanders” that are susceptible to lysis by immune complexes.13,14 Antibodies form against non–RBC-transfused antigens, which lead to the formation of complexes that interact with RBC.

A second proposed mechanism is that activated macrophages destroy RBCs via interactions between certain RBC surface glycoproteins and macrophage adhesion molecule.15 For instance, sickled RBCs express aminophosphatides that enable macrophages to bind to them, subsequently leading to lysis.16 More recent studies show that intercellular adhesion molecule-4, a glycoprotein expressed on RBCs, interacts with macrophages via integrin receptors; transfused RBCs interacting via intercellular adhesion molecule-4 with macrophages are subsequently destroyed by contact lysis or erythrophagocytosis.17

A third proposed mechanism of the anemia in the context of hyperhemolysis is transfusion-induced suppression of erythropoiesis, as an inappropriately low reticulocyte count is commonly seen. However, the usual response to steroids suggests that erythropoietic suppression alone is unlikely responsible for the entire process.18

Treatment of hyperhemolysis is challenging, and the mainstay is to avoid transfusions entirely. Some methods that have been reported to offset hemolysis include steroids, IVIG, and rituximab.19,20

Eculizumab is a humanized monoclonal antibody that inhibits terminal complement activation by targeting the C5 component and blocking its cleavage into C5a and progression to the C5b-9 membrane attack complex. In humans, it is Food and Drug Administration approved for the treatment of patients with paroxysmal nocturnal hemoglobinuria21 and atypical hemolytic nocturnal syndrome.22 Clinical trials have explored eculizumab’s role in treating dense deposit disease and C3 nephropathy, rejection of solid organ transplants, macular degeneration, neuromyelitis optical, myasthenia gravis, dermatomyositis, allergic asthma, antineutrophil cytoplasmic antibody vasculitis, and cold agglutinin disease. We attempted to block hemolysis of transfused RBCs using eculizumab in an off-label fashion selecting an empiric dose in the absence of prior studies. While the drug appears to be effective in reducing the degree of intravascular lysis associated with paroxysmal nocturnal hemoglobinuria, in our case the use of 600 mg IV failed to block hemolysis induced by the RBC alloantibody and hyperhemolysis.

In summary, we report a case of hyperhemolysis in a patient without a Hb disorder that developed after a DHTR due, in part, to an antibody directed against an unknown high-frequency antigen. Treatment with eculizumab did not adequately block intravascular hemolysis and the patient recovered by withholding further transfusions while receiving corticosteroids and EPO. Hyperhemolysis bears some features similar to HLH. Future research may uncover a connection between these two life-threatening hemolytic syndromes.

ACKNOWLEDGMENTS

We specifically thank the members of the Massachusetts General Hospital Blood Transfusion Service and the American Red Cross reference laboratory, who performed the initial and complicated evaluation of the patient’s alloantibodies during Christmas.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES