A case of hyperhemolytic transfusion reaction attributable to anti-Fy3 in a 30-year-old African American woman with a history of sickle cell disease is reported. The patient was admitted for vaso-occlusive sickle cell crisis and received 4 units of packed RBCs secondary to worsening symptomatic anemia (Hb 5.0 g/dL). On admission, the patient's antibody screen and identification showed anti-V and anti-E, and her antibody history included anti-E, -C, -Jkb, -N, -V, -S, and a cold agglutinin with possible anti-I specificity. A DAT performed on her RBCs was negative. RBC units that lacked E, C, Jkb, V, and S were transfused. Posttransfusion Hb was 8.9 g/dL. On day 10 she developed a fever of 103°F and on day 11 her Hb decreased to 6.4 g/dL. She complained of severe back pain and dark urine. In addition, she became hypertensive, tachycardic, and jaundiced. The DAT indicated the presence of IgG on the patient's RBCs. Anti-Fy3 was identified in the serum and eluate. During the next 24 hours, the patient's Hb decreased to 2.4 g/dL. The LDH level was 1687 U/L, and her reticulocyte count was 2.6%. A delayed hemolytic transfusion reaction with hyperhemolysis secondary to anti-Fy3 was suspected and was successfully treated with IVIG and high-dose prednisone.

To the best of our knowledge, this is the first published case of hyperhemolysis in sickle cell disease attributable to anti-Fy3.


Key Words: hyperhemolysis, anti-Fy3, sickle cell disease

Case Report

A 30-year-old African American woman with a history of sickle cell anemia (HbS 83.8%, HbF 11.7%, HbA2 4.5%), hepatitis C secondary to blood transfusion, deep venous thrombosis treated with Coumadin therapy, and previous episodes of acute sickle cell crisis was admitted to our hospital with complaints of fever; shortness of breath; and chest, neck, and back pain which were unresolvable with her usual home medications. At the time of admission, the patient’s Hb was 6.6 g/dL, Hct 20.1%, reticulocyte count 12.9% (corrected, 5.8%), and total bilirubin 2.5 mg/dL (indirect bilirubin, 1.7 mg/dL). Blood cultures were positive for Enterobacter, Klebsiella, and yeast (not Candida albicans). Despite aggressive hydration and initiation of antibiotic therapy, the patient’s clinical status continued to deteriorate. Secondary to worsening anemia with an Hb of 5.0 g/dL, the patient was transfused with 4 units of leukocyte-reduced packed RBCs on hospital days 1 and 2. The patient’s RBCs typed as group O, D+, C-, E-, c+, e+, M+, N-, S-, s+, P1+, K-, Fy(a-b-); and Jk(a+b-). The patient had a previous history of multiple alloantibodies including anti-E, -C, -Jkb, -N, -V, -S, and -Sp and a cold agglutinin with possible anti-I specificity. Of the 4 units issued, 3 units were C-, E-, K-, Fy(a-b-), lifescavig.4-8 Anti-Fy3 is an uncommon antibody that has been described in Fy(a–b–) Caucasians and rarely in African Americans; it is associated with at least one report of an acute hemolytic transfusion reaction.9 Although cases of hyperhemolysis syndrome have been described in the literature, none have been associated with anti-Fy3. This report describes a case of DHT-H attributable to anti-Fy3 in an African American patient with sickle cell disease (SCD).
Jk(b–), S–, and N–. Because of the difficulty of finding phenotypically matched RBCs for the patient, and the urgency with which it was needed, 1 unit that was C–, E–, K–, Fy(a–b+), Jk(b–), S–, and N– was also issued. The patient’s Hb increased to 8.9 g/dL, and her clinical symptoms improved.

On hospital day 10, she developed a fever of 103°F without accompanying symptoms, and blood cultures were negative. By hospital day 11, her Hb decreased to 6.4 g/dL, and she complained of severe back pain and dark urine. In addition she became hypertensive, tachycardic, and jaundiced. The DAT performed on her RBCs was now positive with anti-IgG and negative with anti-C3. As there was clinical suspicion of a delayed hemolytic transfusion reaction, a blood bank investigation was initiated, and further transfusion was withheld. The initial work-up was suggestive of anti-Fy3. This was confirmed by the reference laboratory at Gulf Coast Regional Blood Center in Houston, Texas. Over the course of 24 hours, the patient’s Hb continued to decrease, reaching a nadir of 2.4 g/dL. Her LDH level was 1687 U/L, total bilirubin was 2.7 mg/dL (indirect bilirubin, 1.4 mg/dL), and reticulocyte count was 2.6% (corrected, 0.4%). Haptoglobin measurement was not done. At this point DHTR-H was suspected. Her clinical condition continued to deteriorate, and she was started on high-dose prednisone (60 mg/day) and IVIG (400 mg/kg per day) for a treatment course of 5 days. On hospital day 13 the patient developed clinical symptoms of hemodynamic compromise including shortness of breath, tachypnea, systolic ejection murmur, and severe pallor. Her Hb was 2.6 g/dL. The decision was made to transfuse 1 unit of phenotypically matched (C–, E–, K–, Fy(a–b–), Jk(b–), S–, and N–), leukocyte-reduced packed RBCs and monitor closely for additional signs of hemolysis. Her Hb improved to 3.6 g/dL, and prednisone and IVIG treatment were continued. Her LDH level decreased during the ensuing days, and reticulocyte count increased to 6.3% (corrected, 1.5%). Her Hb continued to improve (up to 5.6 g/dL) and remained stable until hospital day 30 when the patient had an episode of epistaxis because of trauma from her nasal canula and baseline anticoagulation therapy for her history of deep vein thrombosis. Her Hb dropped to 2.6 g/dL. She was transfused with 1 unit of phenotypically matched (C–, E–, K–, Fy[a–b–], Jk[b–], S–, and N–), leukocyte-reduced packed RBCs. After transfusion her Hb increased to 3.3 g/dL and remained stable for the next day. Because her LDH level was 733 U/L and total bilirubin was 6.4 mg/dL with an indirect component of 3.4 mg/dL, hemolysis was again suspected. Her reticulocyte count was 15.1% (corrected, 2.6%) and haptoglobin was less than 6 mg/dL. The patient was restarted on high-dose prednisone (500 mg/day) and IVIG (400 mg/kg/day) immediately for a duration of 5 days. Her hemoglobin and clinical symptoms steadily improved, with no further evidence of hemolytic episodes. She was discharged with a Hb of 6.0 g/dL on hospital day 43. The hemoglobin and reticulocyte counts through her hospital course are shown in Figures 1 and 2.

Materials and Methods

ABO and D testing were performed by standard tube testing using commercial reagents according to the manufacturer’s protocol (Immucor, Inc., Norcross, GA). Antibody detection and identification tests were performed using gel methodology (ID-MTS Gel Test, Ortho-Clinical Diagnostics, Raritan, NJ). The patient’s serum was tested against panels of commercial reagent RBCs (Ortho-Clinical Diagnostics) to determine antibody specificities. The DAT was performed with polyspecific antihuman globulin and monospecific anti-IgG and anti-C3d reagents (Gamma Biologicals, Inc., Houston, TX). The eluate, prepared by an acid elution method (ELU-KIT II, Gamma Biologicals, Inc.) was tested against panels of commercially available reagent RBCs (Ortho-Clinical Diagnostics). The patient’s serum was also tested against ficin-treated RBCs (Ortho-Clinical Diagnostics). The patient’s RBCs were typed for Rh, Kell, MNS, Lewis, Duffy, and Kidd major antigens using specific antisera according to the manufacturer’s protocol (Immucor, Inc., Norcross, GA).

Results

Pretransfusion, the patient typed as group O, D+ with a positive antibody screen. Anti-E and anti-V were identified in the sample. Historic records showed that she had multiple alloantibodies (anti-E, -C, -Jk[b], -N, -V, -S, -I, and -Sla). The phenotype on record was D+, C–, E–, c+, e+; M+, N–, S–, s+; P1+; K–; Fy(a–b–); and Jk(a+b–). The posttransfusion sample showed a positive antibody screen. The patient’s serum was reactive with all Fy(a+) and Fy(b+) RBCs and only nonreactive with a donor RBC that matched her phenotype and was also Fy(a–b–). The serum sample showed reactivity after ficin treatment. The presence of anti-Fy3 was confirmed by the reference laboratory at Gulf Coast Regional Blood Center in Houston, Texas. The DAT was 3+ with polyspecific reagent, 3+ with anti-IgG, and negative with...
Fig. 1. Patient hemoglobin levels throughout hospital admission. PRBCs = packed RBCs.

Fig. 2. Patient reticulocyte counts throughout hospital admission.
anti-C3d. The eluate showed reactivity with most donor RBCs except those that were Fy(a−b−). The phenotypes of the transfused units are as described earlier.

Discussion

This report describes a case of DHTR-H. In this case, a new antibody (anti-Fy3) was detected in the patient's serum on repeat testing. To our knowledge, no case of DHTR-H attributable to the formation of anti-Fy3 alloantibody has been previously published.

Patients with SCD require frequent RBC transfusions or RBC exchange. Alloimmunization to RBC antigens occurs in anywhere from 18 to 47 percent of chronically transfused patients with SCD. The formation of multiple antibodies presents a challenge in finding compatible blood units for these patients, and it is often not possible to provide completely phenotype-matched units and therefore prevent further antibody formation. Delayed hemolytic transfusion reaction (DHTR) is a common complication of alloimmunization. This reaction occurs 3 to 14 days after transfusion and presents with anemia, fever, and a positive DAT. On occasion, hyperhemolysis syndrome may occur as a life-threatening manifestation of DHTR. Hyperhemolysis syndrome is characterized by a drop in posttransfusion hemoglobin below pretransfusion levels, elevated LDH level and bilirubin, fever, pain, and hemoglobinuria. One unique finding in patients with this condition is reticulocytopenia. On many occasions the presenting symptoms of DHTR-H resemble manifestations of sickle cell crises. Therefore, a DHTR must be kept in the differential diagnoses of all recently transfused patients with SCD with vaso-occlusive crises. Delay in diagnosis may lead to inappropriate management and increased morbidity or mortality for these patients. In our case, sickle cell crisis was ruled out, and the presence of a newly detected antibody, combined with a severe drop in hemoglobin levels, supported the diagnosis of DHTR-H.

The mechanism of hyperhemolysis is not completely understood. Multiple studies have supported the fact that autologous RBCs are destroyed, in addition to destruction of transfused RBCs. A bystander hemolysis theory has been proposed. Mechanisms by which this proposed bystander hemolysis occurs include epitope spreading and the defective regulation of the formation of the complement membrane attack complex in sickle RBCs. Sickle RBCs more often expose cryptic antigens and therefore have a high level of surface IgG. This makes the RBCs more susceptible to destruction by active macrophages. Of interest, cases of hyperhemolysis have been reported in thalassemia patients after transfusion of RBC units. This suggests that the presence of sickle RBCs alone is not enough to fully explain the hyperhemolysis phenomenon. In close to 50 percent of cases of hyperhemolysis, no new antibody is identified. Garratty has proposed that in the absence of alloantibodies, other antibodies (including HLA antibodies) reacting with foreign proteins may cause complement activation and RBC hemolysis.

Typically in anemia caused by hemolysis, compensatory reticulocytosis is observed. However, many cases of DHTR-H show inappropriately low reticulocyte counts. The suggested mechanisms of reticulocytopenia include transfusion suppression of erythropoiesis, accelerated destruction of reticulocytes, and decreased levels of erythropoietin secondary to renal damage. Previous case reports have shown an increase in reticulocyte production after the administration of IVIG or steroid treatment. This supports the theory of antibody-mediated accelerated destruction of reticulocytes and hyperactivity of macrophages. In our patient, a drop in the reticulocyte count was observed despite worsening anemia. An increase in the reticulocyte count was observed only after treatment with IVIG and steroids commenced.

In contrast to the DAT in DHTR without hyperhemolysis, the DAT with hyperhemolytic syndrome is most often negative, even in the presence of a new antibody. In a study by Talano et al., patient RBCs in only two of nine episodes of DHTR-H were positive in the DAT. These were both associated with new antibodies (warm autoantibody and anti-E). The negative DAT can be explained by the hemolysis of transfused RBCs. Our patient’s RBCs were negative in the DAT before transfusion, but at the time of the DHTR-H, the DAT was positive and anti-Fy3 was eluted from RBCs. However, the DAT was not repeated when her hemoglobin dropped further. The DAT remained positive even after the second episode of hyperhemolysis that occurred 3 to 4 weeks later with a negative eluate. Our findings and the results of others show that the DAT cannot not be used alone to diagnose DHTR-H.

Anti-Fy3 has been reported to cause both acute and delayed hemolytic transfusion reactions. Several RBC antibodies have been implicated as a cause of DHTR-H, but there are no published reports of anti-Fy3 as a cause of such reactions. This antibody has been described in individuals with the Fy(a−b−) phenotype, but it very
rarely occurs in African Americans. Anti-Fy3 was first described in a Caucasian Australian woman whose RBCs were typed as Fy(a–b–). This antibody reacted with all RBCs that were Fy(a+b–), Fy(a+b+), and Fy(a+b+). Unlike anti-Fya and anti-Fyb, anti-Fy3 reacted with enzyme-treated RBCs. Considering that the Fy(a–b–) phenotype is prevalent in the West African and African American populations, one would expect that anti-Fy3 would be a commonly encountered antibody. However, anti-Fy3 is rarely encountered, and the majority of cases have been reported in Caucasians. The molecular mechanisms behind the Fy(a–b–) phenotype in West African and Caucasian individuals differ. It is believed that most Africans are homozygous for an FYB allele with a point mutation in the promoter region of the gene that prevents expression of the Duffy antigens on RBCs. However, it has been shown that Fyb is expressed on tissue cells of these same individuals, particularly the endothelial cells of postcapillary venules and Purkinje cells of the cerebellum. Therefore, they are prevented from recognizing Fyb as foreign and from forming anti-Fyb or anti-Fy3. In Caucasians, it has been found that the Fy(a–b–) phenotype is the result of a deletion in the FY genes, and therefore there is a lack of expression of Duffy antigens on their RBCs and tissue cells. These individuals are capable of forming antibodies to all of the Duffy antigens, including Fy3. Although this genetic mechanism is known to be found mostly in Caucasians, it has been proposed that both genetic mechanisms exist in the African population. Our patient is African American and therefore would not have been expected to make this antibody. Genotyping studies could not be performed, but it is proposed that her Fy(a–b–) phenotype is the result of a deletion in FY genes rather than a defect in the FY promoter. When one is looking for phenotypically matched RBCs for African American patients with the Fy(a–b–) phenotype, often the Fy(b–) requirement will be omitted to find units more quickly and because of the improbability of anti-Fyb formation in these patients. However, in our patient, giving a Fyb(+)(b+) RBC unit likely contributed to the initiation of DHTR-H.

Interestingly, this patient also had a history of anti-Sp, which had been identified by another hospital approximately 10 years earlier. Anti-Sp and anti-Fy3 can be difficult to distinguish serologically, and RBCs that are Fy(a–b–) also tend to be Sp(a–). However, whereas anti-Sp reactivity is depressed with ficin treatment of RBCs, anti-Fy3 activity is unaffected by enzyme treatment. This can be an important distinguishing feature when differentiating between these antibodies. In our patient, our reference laboratory (Gulf Coast Regional Blood Center, Houston, TX) did not identify anti-Sp after multiple and extensive evaluations.

In patients with DHTR-H, transfusion of more blood may exacerbate hemolysis. Therefore the transfusion of RBCs is discouraged unless severe, life-threatening anemia occurs. In our patient, transfusions were withheld until the patient developed severe, symptomatic anemia. She appeared to have responded to IVIG and steroid treatment with an increasing hemoglobin and reticulocyte count. However, after a second transfusion, hyperhemolysis again occurred. Many studies have reported success with high-dose IVIG and corticosteroids, either alone to prevent hemolysis or in conjunction with RBC transfusions. How these drugs help reduce hemolysis is not clear, but some of the proposed mechanisms include (1) IVIG prevention of contact-mediated lysis of the RBCs by blocking the adhesion of sickle cells, reticulocytes, and macrophages, and (2) IVIG and steroid suppression of hyperactive macrophages. Additional trials and studies are needed to further define the role of these drugs in this condition. Although transfusion precipitates most cases of hyperhemolysis in patients with SCD, there have been reports of this condition occurring in patients with uncomplicated acute painful episodes. It is possible the patient’s underlying SCD and acute painful crisis, even without transfusion, could have contributed to the hyperhemolytic crisis.

Whether this reaction will occur again in patients who have had one episode of DHTR-H is not clear. Our patient seems to be one in whom DHTR-H will very likely recur. This is despite attempts to provide blood that matched her complete phenotype. Other reports have also shown that giving phenotypically matched blood did not prevent the occurrence of DHTR-H in some patients. Since this episode, this patient has presented to the hospital multiple times but has not been transfused and has maintained baseline Hb of 5 to 6 g/dL.

**Conclusion**

In conclusion, this is a report of DHTR-H attributable to anti-Fy3 in an African American woman with SCD. Anti-Fy3, although rare in African Americans and West Africans, can be associated with hyper-hemolysis. This report shows that hyperhemolysis is a serious complication that should be sought for early and that providing phenotypically matched units will not always prevent...
DHTR-H from occurring. Withholding trans-fusions and initiating treatment with IVIG and steroids may be lifesaving. The complete pathogenesis of this condition and other treatment options available continue to be explored.

References

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