Case Report

Hematologic complications in a patient with *Glycine soja* polyagglutination following fresh frozen plasma transfusion

R.P. Jajosky, L.O. Cook, E. Manaloor, J.F. Shikle, and R.J. Bollag

Polyagglutination is a rare and underdiagnosed condition, characterized by agglutination of red blood cells (RBCs) with almost all ABO-compatible adult sera. Polyagglutination can occur when a cryptantigen is exposed on RBCs via microbial enzyme activity. Because nearly all adults naturally produce antibodies against cryptantigens, transfusion of plasma can cause unexpected hemolysis and hematologic complications, such as thrombocytopenia and disseminated intravascular coagulation, in patients whose cryptantigens are exposed. We report a case of *Glycine soja* polyagglutination occurring in a 60-year-old African-American man with disseminated methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Prior to transfusion, the patient developed severe anemia of unknown etiology. Following transfusion of 3 units of fresh frozen plasma (FFP), his RBC count could not be determined for 24 days because of RBC agglutination in his blood sample. In addition, the FFP transfusion correlated with the rapid development of severe, transfusion-refractory thrombocytopenia and anemia. The perplexed clinical team consulted the blood bank. A direct antiglobulin test demonstrated 1+ mixed-field reactivity with both monoclonal anti-IgG and anti-C3d. Lectin panel testing showed reactivity with only *Glycine soja,* confirming the condition. Subsequently, plasma components were avoided, and RBC and platelet (PLT) components were washed prior to transfusion. After a 44-day hospitalization involving the transfusion of 22 units of RBCs and 13 units of PLTs, the patient was discharged to a long-term care facility. The patient's confounding hematologic complications can best be explained by polyagglutination, which developed secondary to the severe MRSA infection. The FFP transfusion likely passively transferred antibodies that bound to the patient's RBC cryptantigens, leading to RBC agglutination and anemia. The development of severe thrombocytopenia may be related to cryptantigen exposure on the patient's PLTs. Although difficult to identify, polyagglutination needs to be recognized to appropriately manage hemotherapy. The purpose of this case study is to report hematologic complications following FFP transfusion in a patient with *Glycine soja* polyagglutination, a rarely described condition.
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Polyagglutination describes the agglutination of red blood cells (RBCs) that occurs with nearly all compatible adult sera.[1,2] It arises through an alteration of glycoprotein moieties on the RBC membrane. This condition is usually secondary to infection[1,2] rather than to a congenital or somatic mutation. T activation is the most common form of microbial-induced polyagglutination and serves as a prototypical example (Fig. 1).[3] Antibodies against cryptantigens are naturally occurring and are usually IgM.[3] The patient's own antibodies may mediate hemolysis.[4] However, it is more common for hemolysis to occur after the transfusion of blood components that contain plasma.[5–7] Thrombocytopenia may develop because of the presence of the cryptantigen on platelets (PLTs).[8,9] In addition, coagulopathies, such as disseminated intravascular coagulation (DIC), often occur.[5,10]

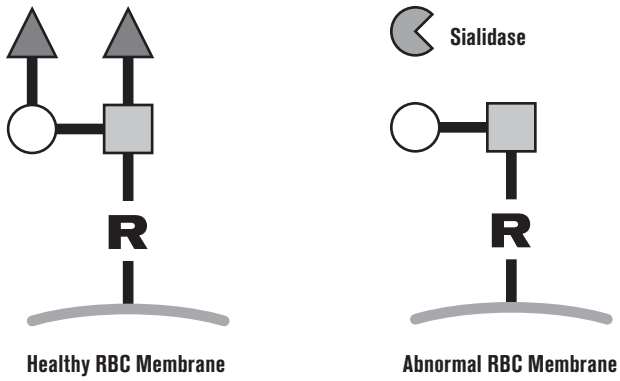

Fig. 1 Microbial enzymes such as sialidases (circle sector shape) can enzymatically remove *N*-acetylneuraminic acid (triangles) from RBC antigens. This action exposes normally hidden cryptantigens, such as T (shown on the right), which can subsequently bind anti-T. This particular type of polyagglutination is known as T activation. This form is often described in pediatric patients with necrotizing enterocolitis or atypical hemolytic uremic syndrome caused by *Streptococcus pneumoniae.* T is also present on platelets and glomerular endothelial cells. The circle designates D-galactose. The square designates *N*-acetyl-D-galactosamine. R = Remainder of molecule; RBC = red blood cell.

In the past, polyagglutination was readily detected by blood centers when ABO typing resulted in ABO discrepancies.[8,11] This finding occurred because human-source sera containing antibodies against cryptantigens were used for blood typing. The transition to monoclonal antibodies for blood typing

eliminated these ABO discrepancies. In addition, most blood centers do not routinely screen for polyagglutination.[12] Therefore, polyagglutination is becoming under-recognized. To identify polyagglutination, the condition must be suspected by either clinicians or blood bank personnel, and additional testing must then be performed. Additional features seen in polyagglutination include a C3-positive direct antiglobulin test (DAT) and/or a reverse ABO typing discrepancy.[1,8]

Lectin panel testing should be used to properly identify and classify polyagglutination.[1,2] Lectins are proteins that bind to carbohydrate antigens.[13] Lectin panel testing is not available in most blood banks, although this testing is performed by reference laboratories. Alternatively, polyagglutination can be more easily detected by demonstrating RBC agglutination with nearly all compatible adult sera. In rare cases, RBCs from patients with polyagglutination are not truly polyagglutinable, and polyagglutination can only be diagnosed using lectins.[2]

Suspected polyagglutination may prompt the use of specialized transfusion protocols to minimize the passive transfer of antibodies targeting cryptantigens. Protocols involve avoiding plasma components and washing units of RBCs and PLTs.[12,14] If plasma components must be given, the least incompatible units, as determined by minor crossmatch, should be selected. In addition, blood components should be transfused slowly with careful monitoring of the patient. For critically ill patients, plasma exchange using albumin as the replacement fluid[9] and RBC exchange using washed RBCs[12] have been reported as beneficial. Nevertheless, these transfusion practices are not universally accepted because of skepticism about a causal relationship between polyagglutination and hemolysis.[3,12] In addition, some physicians note risks associated with avoiding therapeutic blood components and with the time delay caused by washing. In addition, washing can lead to bacterial contamination, the loss of 20 percent of RBCs, the loss of 25 percent of PLTs, and impaired hemostatic function of PLTs.[14] Also, reports of uneventful transfusion of plasma components[15] have led some physicians to discount the potential risks of transfusion.

Case Report

A 60-year-old African-American man with a past medical history of epileptic seizures developed methicillin-resistant *Staphylococcus aureus* (MRSA) endocarditis involving the free wall of the right atrium, with secondary seeding of the left knee. The patient was treated with intravenous antibiotics (vancomycin and piperacillin-tazobactam) and underwent two incision and drainage (I&D) procedures of the septic knee. Subsequently, his hemoglobin declined from 11.4 to 7.1 g/dL (reference range: 14.0–18.0 g/dL) for unknown reasons (Fig. 2). The patient's blood typed as group A, D+, and multiple RBC units were transfused. Before the third I&D procedure, the patient received 3 units of fresh frozen plasma (FFP) for reversal of warfarin, which was being given for a thrombus that formed over the cardiac vegetation.

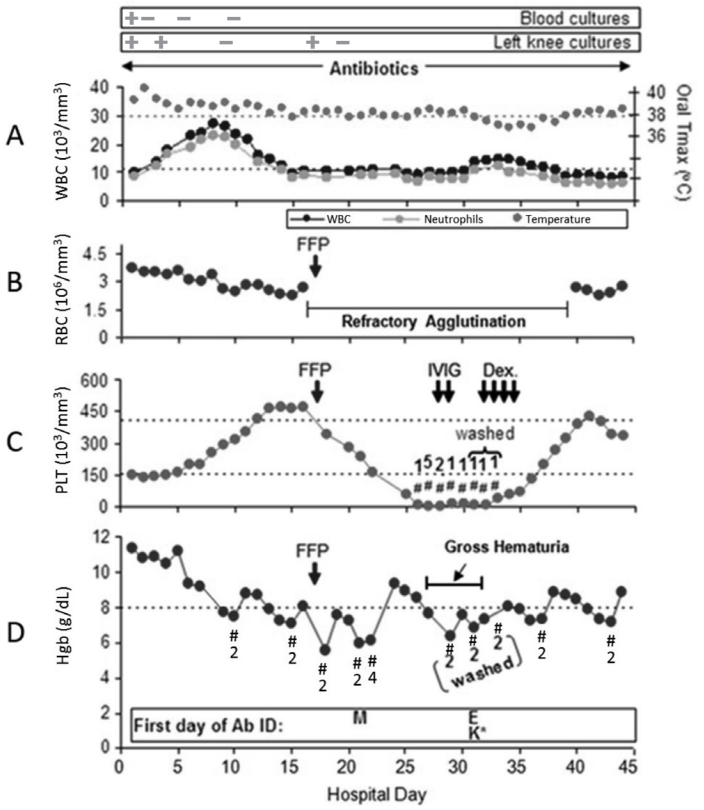

Fig. 2 Hematology values throughout the hospital course. (A) The WBC count peaked early in the hospital stay. The patient remained febrile throughout the hospitalization, however, developing disseminated infections involving the lungs, soft tissues, and bones. (B) The RBC count, including the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW), could not be reported for 24 days after the transfusion of FFP because of agglutination in the patient's blood sample, refractory to 37°C warming. (C) Mild thrombocytosis developed into severe thrombocytopenia over 9 days after the transfusion of FFP. (D) The persistently declining hemoglobin was managed with numerous RBC transfusions throughout the hospitalization, several of which were with washed RBC units. Anti-M and anti-E were subsequently identified; anti-K could not be excluded. WBC = white blood cell; Tmax = maximum temperature; RBC = red blood cell; FFP = fresh frozen plasma; PLT = platelet; IVIG = intravenous immunoglobulin; DEX = dexamethasone; # = number of units transfused; Hgb = hemoglobin; Ab ID = antibody identification. *Anti-K could not be excluded.

Following the FFP transfusion, the RBC indices could not be reported because of RBC agglutination in the patient's blood sample (Fig. 3), refractory to 37°C warming. Peripheral blood smears revealed large irregular clusters of RBCs, consistent with agglutination rather than rouleaux. No spherocytes or schistocytes were identified. After the FFP transfusion, his hemoglobin dropped from 8.1 to 5.6 g/dL. Upon retyping, the RBCs were 4+ reactive with anti-A reagent (Gamma-clone, ImmucorGamma, Norcross, GA) and weakly reactive with anti-B reagent (Gamma-clone, ImmucorGamma), representing a forward ABO type discrepancy. In addition, the patient's sera was 3+ reactive with B cells (Referencells, ImmucorGamma) and weakly reactive with A_1 cells (Referencells, ImmucorGamma). The reactivity with reagent A_1 cells was not previously seen. The antibody screen was negative when using a 45-minute pre-warm method without polyethylene glycol enhancement.

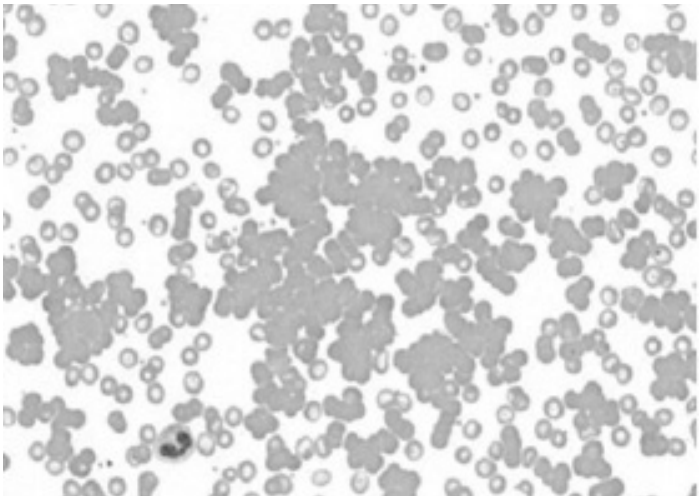

Fig. 3 The peripheral blood film from the day after the transfusion of fresh frozen plasma showed large aggregates of erythrocytes not seen previously. Subsequent blood films showed progressively smaller aggregates and the development of severe thrombocytopenia. Blood films consistently showed neutrophils with toxic changes and few polychromatophilic macrocytes, but no spherocytes, schistocytes, or platelet aggregates were seen.

To address the unexplained anemia, 8 units of RBCs were transfused over 5 days. Because the clinical team suspected bleeding rather than hemolysis, relevant laboratory values were only obtained at the beginning of this episode. These labs demonstrated a total bilirubin of 1.3 mg/dL (reference range: 0.3–1.2 mg/dL); lactic acid dehydrogenase (LDH) of 377 U/L (reference range: 120–246 U/L); and a haptoglobin of 177 mg/dL (reference range: 40–240 mg/dL), which was greater than 340 mg/dL prior to the FFP transfusion. A fecal occult blood test and urinalysis did not identify a source of bleeding. In addition, a computed tomography scan revealed only a small hematoma of the left thigh. The FFP transfusion also correlated with a rapidly declining PLT count, which was 472,000/mm^3 (reference range: 150,000–400,000/mm^3) prior to transfusion, and 8000/mm^3 9 days later. DIC was not favored as contributory to the thrombocytopenia because of the absence of schistocytes on peripheral blood films. Heparin was discontinued, even though the anti-platelet factor 4/heparin antibody test was negative. Perplexed by the hematologic complications, the clinical team consulted the blood bank.

The transfusion medicine service considered polyagglutination in the differential diagnosis. A DAT was performed and demonstrated 1+ mixed-field reactivity with polyspecific anti-human globulin (AHG), monoclonal anti-IgG, and anti-C3d. Next, a sample with a request for lectin panel testing was sent to a reference laboratory. Chloroquine-treated patient RBCs were weakly reactive with four sources of group AB plasma/sera at albumin (ALB)-37°C phase, including the diluent control, but were nonreactive at immediate spin (IS), room temperature incubation (RT), and ALB-IgG AHG phases. Patient plasma reacted with group A_1, A_2, and B reagent RBCs. In addition, the RBCs demonstrated reactivity with *Glycine soja,* but not *Arachis hypogaea, Salvia horminum,* or *Salvia sclarea*. This constellation of reactivity represents an extremely rare form of polyagglutination, not formally classified.

The blood bank informed the clinical team that plasma components should not be transfused and that units of RBCs and PLTs should be washed. Thereafter, the patient received multiple units of washed RBCs, although some units could not be washed because of staffing issues. The washed units of RBCs did not increase the hemoglobin by the expected increment. The patient developed anti-M and anti-E; anti-K could not be excluded (Fig. 2). All units of RBCs transfused during the hospitalization were AHG crossmatch-compatible. The severe thrombocytopenia was unresponsive to washed PLT transfusions. A course of intravenous dexamethasone resulted in concurrent resolution of the thrombocytopenia. After a complicated 44-day hospital course, the patient was transferred to a long-term care facility in stable condition. Since recovering from the infective endocarditis, the patient continues to see the neurology service at our hospital for treatment of his seizure disorder.

Discussion

The purpose of this case study is to report on *Glycine soja* polyagglutination, a rarely described condition that has not been associated with infection. Polyagglutination is a rare condition that can be caused by cryptantigen exposure on RBCs through microbial enzyme activity. Because antibodies against cryptantigens are naturally occurring, transfusion of blood components, which contain plasma, can cause hematologic complications. The identification of polyagglutination involves lectin panel testing, which is available at reference laboratories but not available at most hospital transfusion services. Nevertheless, it is important to diagnose polyagglutination because patients with this condition are at risk for complications following the transfusion of plasma-containing blood components. Therefore, if possible, plasma components should be avoided, and units of RBCs and PLTs should be washed to avoid the passive transfer of antibodies targeting cryptantigens.

This report describes a 60-year-old African-American man who was found to have *Glycine soja* polyagglutination after developing a severe MRSA infection. Although the patient's clinical course was complex, the patient's history can best be explained by complications of polyagglutination. Prior to transfusion, the patient developed severe anemia of unknown etiology, possibly due to endogenous antibodies binding to the cryptantigens. The passive transfer of donor antibodies, targeting cryptantigens, can explain the onset of severe RBC agglutination, the forward ABO typing discrepancy, and the transfusion-refractory anemia. The reverse ABO typing discrepancy can best be explained by anti-M, which was first identified 3 weeks post-admission. The severe thrombocytopenia that rapidly developed after the FFP transfusion can be explained by cryptantigen exposure on the PLTs. Unfortunately, transfused units of washed RBCs and PLTs did not provide the increase in cell counts that was expected. This finding may be due to in vivo cryptantigen exposure on the transfused RBCs and PLTs.

Alternative diagnoses do not fully explain the patient's complex hematologic findings. Post-transfusion purpura (PTP) can account for the severe thrombocytopenia (PLT <10,000/mm^3), as this occurred 9 days after the FFP transfusion.[16] PTP is mediated by antibodies against class II HLA molecules or PLT-specific antigens, of which antibodies to human platelet antigen (HPA)-1a are most common.[16] Alternatively, drug-induced thrombocytopenia, caused by vancomycin, could explain the severe thrombocytopenia.[16] Studies to identify antibodies to HPA were not conducted, so PTP could not be formally excluded. However, neither PTP nor drug-induced thrombocytopenia is associated with RBC agglutination, anemia, or *Glycine soja* lectin reactivity. Drug-induced immune hemolytic anemia, due to piperacillin, can account for the positive DAT and anemia,[16] although it cannot explain the severe thrombocytopenia or *Glycine soja* reactivity. A combination of drug-induced immune hemolytic anemia with PTP or drug-induced thrombocytopenia is unlikely, given that the onset of RBC agglutination and the rapid and severe decline in PLT count were temporally concurrent with the transfusion of FFP.

Glycine soja polyagglutination is distinct from other microbial-induced forms such as T activation (the most common form) or Th (an incomplete form of T activation), Tk, and Tx polyagglutination. Other unique features of *Glycine soja* polyagglutination include its association with *S. aureus,* RBC agglutination refractory to 37°C warming, and thrombocytopenia occurring in the absence of DIC.

Reports of *Glycine soja* polyagglutination have been described. A prospective study screened patients at high risk for polyagglutination with the soybean lectin *Glycine soja* and the peanut lectin *Arachis hypogaea*.[17] Two out of 238 patients were identified with RBCs that reacted only with *Glycine soja*. One patient was a 71-year-old man with immunoblastic lymphoma. The other was a 52-year-old woman with a paravertebral mass. The authors included these two patients among a total of 18 with reported cryptantigen exposure. The study also examined 302 healthy adults in the control group and found no lectin reactivity among these patients. Another report of *Glycine soja* polyagglutination described a healthy blood donor from Bermuda whose RBCs demonstrated agglutination with the majority of recipient sera.[18] Interestingly, the agglutination was refractory to 37°C warming. Further studies revealed that this form of polyagglutination was related to an inherited form known as Cad, which represents the strongest expression of Sd^a. The authors named this form Cad_{Ber} (Ber = Bermuda).

Conclusions

Polyagglutination is a rare, potentially fatal condition that may occur in septic patients, causing confounding anemia, thrombocytopenia, and DIC. These complications may occur in nontransfused patients but are more common in patients receiving plasma-containing blood components. Polyagglutination can be detected by demonstrating RBC agglutination with nearly all compatible adult sera, and lectin panel testing can confirm the suspected diagnosis. Transfusion protocols such as avoiding plasma components and the use

of washed units of PLTs and RBCs are indicated to avoid potentially serious complications. Resolution of the underlying infection should lead to resolution of polyagglutination.

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References

1. Horn KD. The classification, recognition and significance of polyagglutination in transfusion medicine. Blood Rev 1999;13:36–44.
2. Beck ML. Red blood cell polyagglutination: clinical aspects. Semin Hematol 2000;37:186–96.
3. Eder AF, Manno CS. Does red-cell T activation matter? Br J Haematol 2001:114:25–30.
4. Rickard KA, Robinson RJ, Worlledge S. Acute acquired haemolytic anaemia associated with polyagglutination. Arch Dis Child 1969;44:102–5.
5. Wang Q, Liu D, Bai Y. T-cryptantigen (TCA) activation in severe pneumonia complicated with multiple organ failure. Transfus Apheresis Sci 2010;43:361–4.
6. Bird T, Stephenson J. Acute haemolytic anaemia associated with polyagglutinability of red cells. J Clin Pathol 1973;26:868–70.
7. Levene C, Sela R, Blat J, et al. Intravascular [correction of intracellular] hemolysis and renal failure in a patient with T polyagglutination. Transfusion 1986;26:243–5.
8. Cochran J, Panzarino V, Maes L, et al. Pneumococcus-induced T-antigen activation in hemolytic uremic syndrome and anemia. Pediatric Nephrol 2004;19:317–21.
9. Hopkins C, Yuan S, Lu Q, et al. A severe case of atypical hemolytic uremic syndrome associated with pneumoccal infection and T activation treated successfully with plasma exchange. Transfusion 2008;48:2448–52.
10. Boralessa H, Modi N, Cockburn H, et al. RBC T activation and hemolysis in a neonatal intensive care population: implications for transfusion practice. Transfusion 2002;42:1428–34.
11. Ramasethu J, Luban N. T activation. Br J Haematol 2001;112:259–63.
12. Crookston KP, Reiner AP, Cooper LJN. RBC T activation and hemolysis: implications for pediatric transfusion management. Transfusion 2000;40:801–12.
13. Bird GWG. Lectins in immunohematology. Transfusion Med Rev 1989;3:55–62.
14. Wang LY, Chan YS, Chang FC, et al. Thomsen-Friedenreich activation in infants with necrotizing enterocolitis in Taiwan. Transfusion 2011;51:1972–6.
15. Eversole M, Nonemaker B, Zurek K, et al. Uneventful administration of plasma products in a recipient with T-activated red cells. Transfusion 1986;26:182–5.
16. Fung MK, Grossman BJ, Hillyer CD, et al. AABB technical manual. 18th ed. Bethesda, MD: AABB, 2014.
17. Buskila D, Levene C, Bird GWG. Polyagglutination in hospitalized patients: a prospective study. Vox Sang 1987;52:99–102.
18. Leger R, Lines E, Cunningham K, Garratty G. A new form of polyagglutination related to Cad. Immunohematology 1996;12:69–71.

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