Report

The prevention and management of alloimmunization in sickle cell disease: the benefit of extended phenotypic matching of red blood cells

E.P. Vichinsky

The Northern California Sickle Cell Center at Children's Hospital Oakland offers comprehensive pediatric and adult services to 700 active patients with sickle cell disease (SCD). This includes 6900 outpatient visits and approximately 5000 inpatient days with an average length of stay of 4.7 days. Additionally, this includes a reference hemoglobin biology laboratory, a stem cell transplantation program, a 20-bed pheresis transfusion unit, an ambulatory center, and a centralized inpatient floor. Specialized services for nutrition, physiatry, pulmonary disease, brain function, transition program for adolescents, mental health services, and transfusion are included in our core program. The program focuses on prevention and treatment of complications. Prevention and treatment of brain injury is a major focus of the program. Approximately 6 percent of our annual transcranial Doppler ultrasonographs (TCDs) are abnormal, requiring transfusion therapy, and 16 percent of conditional TCDs eventually have converted to abnormal in our program, resulting in transfusion therapy.[1] Preliminary studies suggest children with asymptomatic neuroimaging abnormalities have neurocognitive injury and both progress with time. Transfusion therapy is being studied to prevent this progression presently. Although more than 100 patients are receiving hydroxyurea therapy in our program, transfusion therapy remains a core treatment for many patients.

Chronic transfusion therapy is recommended for the prevention and for the treatment of many complications of SCD.[1] Patients are transfused in a 17-bed ambulatory transfusion unit with weekend availability. All transfused patients are followed in a comprehensive transfusion program that offers red blood cell (RBC) pheresis, quantitative monitoring of body iron stores, and access to multiple iron chelators. Sixty pediatric patients and 40 adults are receiving chronic transfusions, with 40 patients receiving chronic RBC pheresis.

Transfusion Protocol

All patients upon enrollment in our program and before transfusion therapy undergo detailed pretransfusion testing for RBC antigens by serology and genotyping. Such genotyping has resulted in improved matching of individuals. Since 1994, all patients received blood matched for C, c, E, e, and K in addition to ABO and D. Once an antibody was made, phenotypic matching was extended to include Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, and N. All patients commencing chronic transfusion have antibody formation and reactions monitored and recorded on a monthly basis. Each transfusion is preceded by a complete blood count, antibody screening, and hemoglobin S level. Patients initially undergo a routine antibody screening with a three-cell panel using a gel method (Ortho ID-MTS, Ortho Clinical Diagnostics, Rochester, NY). Positive screens trigger antibody identification with a ten-cell panel. When possible, absorption methods to detect autoantibodies are performed. Any antibodies with complex specificities are sent to the Blood Bank of the Pacific.

To maintain an extended phenotyping RBC transfusion program for patients with SCD, a minority donor recruitment program is supported. This program focuses on recruiting minority donors at alternative sites, such as churches, hospitals, and community events. Ethnic matching of units is not performed; however, all units are screened for sickle cell trait by solubility testing. Units are stored in CPDA-1 or Adsol S-1 (Baxter Corp., Round Lake, IL). There is no age requirement for the donor blood unit beyond standard life guidelines except in selected cases. Clinical data are developing that support the use of fresh blood. We are attempting to provide units less than 2 weeks old, but this is very difficult and has not been a requirement for most patients. All units undergo leukocyte reduction at the blood bank. Therefore, cytomegalovirus (CMV) screening, as well as irradiation of units, is not used except in the immediate pretransplant

management of patients. Although many programs irradiate RBCs, there is no evidence of benefit to patients with SCD, and there is a suggestion of shortened RBC survival.

Methods of Transfusion

Simple transfusions in the ambulatory setting, designed to maintain a pretransfusion hemoglobin (Hb) greater than 9 g/dL, is our standard protocol.[2] When hemoglobin S (HbS) levels are high, the maximum Hb is maintained at less than 11 g/dL because of the risk of hyperviscosity. In chronically transfused patients with low HbS levels, maximum Hb levels as high as 12 g/dL are maintained. RBC pheresis is routinely used for initial stroke management and in the care of patients with high baseline Hb or difficulty in maintaining iron balance with iron chelation therapy.[3,4]

Children's Hospital Oakland Experience

Since 1990, we have prospectively been following the efficacy and complications of a chronic RBC transfusion. In 1992, we initiated a mandatory leukoreduced RBC program, and in 1994, we initiated a mandatory extended RBC matching for ABO, D, Cc, Ee, and K. In this program, once an antibody was formed, patients received units fully matched for Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, and N. On rare occasions, patients who were unable to receive phenotypically matched units because of emergencies were transfused with D– K– RBC units. None of these patients developed antibodies. Before the initiation of phenotypically matched units, a retrospective review of our data indicated an alloimmunization rate of approximately 2 percent to 3 percent per unit of exposure. After initiation of our matching program, the overall alloimmunization rate fell to 0.05 antibodies per 100 units transfused.[5] The alloantibodies that developed were anti-Le^a, -M, -D, -C, -Kp^a, and -rh_i. The workup suggested one patient was a partial D who typed as D+. At least three of these patients were transfused at outside institutions transiently. No patients developed progressive alloimmunization. Three autoantibodies occurred. These were warm IgG antibodies. All three autoantibodies were transient and did not prevent transfusion. We used the least incompatible units with complete phenotyping, and there was no evidence of hemolysis. In contrast, we did experience, in thalassemia, two patients with autoimmune hemolytic anemia that was associated with prior alloimmunization. Both patients were alloimmunized before enrolling in our program. One patient responded completely to Rituximab. The other patient showed transient elimination of the antibody when rechallenged with phenotype-matched units. This patient successfully underwent a stem cell transplantation using Alemtuzumabe (Campath, Genzyme, Inc., Cambridge, MA) which resulted in complete elimination of the autoantibody. Before our matching program, hemolytic transfusion reactions occurred in 11 percent of our chronically transfused population. After the initiation of our extended RBC matching program, we have experienced only one hemolytic reaction. This patient was noted to have a lack of response to transfusion with a rising HbS level. The workup identified anti-Le^a and -M, two antibodies that are generally not associated with hemolysis. The patient has been transfused for several years after this event and has not developed any transfusion complications.

Management of Complications

Prevention of Transfusion Reactions

Alloimmunization is one of the most important complications of transfusion therapy and SCD.[6,7] The risk of alloimmunization to RBC antigens varies among individuals. There clearly are genetic determinants of patients with increased rate of antibody formation. Clinical and experimental data indicate that HLA molecules, proinflammatory cytokines (e.g., interleukin [IL] 1, IL-6, IL-8), costimulatory molecules (e.g., IL-10, transforming growth factor), and signaling molecules (e.g., cytotoxic T-lymphocyte antigen 4) associated with CD4-regulatory T cells mediate the response to alloimmunization.[8,9] In addition, factors such as splenectomy, patient age, and disease subtype appear to be important. The two most important factors responsible for alloimmunization in patients with SCD are the number of transfusion exposures and the disparity in RBC phenotype between donor and recipient.[10–12] With standard RBC matching, eventually 40 percent to 80 percent of recurrently transfused adult sickle cell anemia patients are alloimmunized.[10] Our first approach to this problem is using limited and extended RBC phenotyping.

Many of the antibodies are not persistent, and patients are receiving transfusions at multiple locations without information about their prior transfusion records.[10] Unfortunately there is no central blood bank registry for patient reactions. The transfusion of RBCs to a patient previously sensitized to a transient clinically significant antigen is one of the most common causes of transfusion reactions. To address this, each patient is given a card or letter with his or her RBC phenotype and transfusion history. The patient is educated to present this information at each facility.

To prevent alloimmunization, all patients with SCD undergo complete antigen RBC typing. The RBC phenotype

is augmented by a genotype in patients who are chronically transfused.[13] The genotype results enable the identification of patients with hybrid alleles at risk for undetected antibody reactions.[14,15] For example, altered C antigens are present in as many as 20 percent of African Americans. These patients type serologically as C+ but make anti-C.[16] Patients without a history of immunologic reactions receive prospectively matched units for C, E, and K, as well as high-risk mutations uncovered with genotype testing.[14] Once a clinically significant antibody develops, patients undergo extended matching. Duffy, Kidd, Lewis, and MNS are included. The reference blood bank is incorporated in a prospective transfusion regimen to plan for blood transfusion requests.

Autoantibodies

A positive direct antiglobulin test (DAT) is a common complication in transfused patients with SCD that results in significant clinical, laboratory, and financial problems.[4,17] Inflammation, existing alloantibody, and genetic polymorphisms are risk factors. A positive DAT needs to be evaluated in the clinical setting. This includes laboratory evaluation for evidence of increased hemolysis and a detailed review of the transfusion history to rule out a primary immunization. A review of the patient's drug history should be performed. Drugs such as cephalosporins are associated with positive DATs but rarely with hemolysis. Detailed evaluation of the positive DAT for IgG complement, strength, and temperature are necessary. Elution of the antibody and evaluation for a masked alloantibody are necessary. In general, the strength of the DAT does reflect the risk of hemolysis. Nonspecific, weakly positive DATs are commonly stimulated by chronic transfusions and are usually transient. Many patients have a polyspecific positive DAT that is negative on follow-up evaluation with monospecific reagents. Occasionally, they do have specificity. Our approach is to aggressively evaluate the patients with a positive DAT, including elution and specificity. If the patient shows no evidence of hemolysis or the DAT is weak, we continue the transfusion program using phenotypically matched units.

Hemolytic Transfusion Reaction With Negative Evaluation

Patients with SCD are prone to DAT-negative hyperhemolytic crisis.[18,19] This occurs usually in chronically transfused patients who have clinical findings during an acute illness consistent with a hemolytic transfusion reaction. The cause of this serious event is unknown. Older, stored donor RBCs may accelerate hemolysis in patients with SCD by eryptosis with phosphatidylserine activation of an inflammatory hemolytic process. Clinicians have documented a bystander effect in which hemolysis of the transfused units induces hemolysis of the patient's RBCs. Treating severe hemolytic transfusion reactions, whether DAT positive or negative, is difficult. Our approach is to avoid transfusions and use conservative therapy if possible. Lifesaving transfusions should not be withheld. Support of these patients is possible. We have used intravenous immunoglobulin (IVIG) therapy, high-dose erythropoietin, and steroids. Our success has been limited. Recently, we have found Rituximab to be effective in modulating the hemolysis.[20]

In summary, transfusion therapy is increasingly used despite advances in treatment of SCD. As patients age, transfusion exposure significantly increases. Transfusion complications can be largely prevented by implementation of preventive strategies.[21,22] These are not routinely implemented, largely because of concerns for cost. Detailed studies comprehensively evaluating the risk and benefit, focusing on cost, have not been completed.[23] Several programs describe cost savings based on decreased need to search for specialty units because of fewer antibodies. Others have indicated the cost decreases because of the expansion of minority recruitment and the increased availability of rare antigen donors.[24] However, patient safety should supersede cost savings. Therefore, our approach has been to focus on decreasing clinical morbidity. We have previously demonstrated that phenotypically matched units dramatically decrease the rate of alloimmunization. We are presently working to expand our donor pool, evaluate the benefit of genotyping, and identify clinically useful genetic risk factors for transfusion reactions.

References

1. Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med 1998;339:5–11.
2. Haberkern CM, Neumayr LD, Orringer EP, et al. Cholecystectomy in sickle cell anemia patients: perioperative outcome of 364 cases from the National Preoperative Transfusion Study. Preoperative Transfusion in Sickle Cell Disease Study Group. Blood 1997;89:1533–42.
3. Hulbert ML, Scothorn DJ, Panepinto JA, et al. Exchange blood transfusion compared with simple transfusion for first overt stroke is associated with a lower risk of subsequent stroke: a retrospective cohort study of 137 children with sickle cell anemia. J Pediatr 2006;149:710–12.
4. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. Blood 2000;96:3369–73.

5. Wahl S, Garcia A, Hagar W, Gildengorin G, Quirolo K, Vichinsky E. Lower alloimmunization rates in pediatric sickle cell patients on chronic erythrocytapheresis compared to chronic simple transfusions. Transfusion, 2012. In press.
6. Afenyi-Annan A, Willis MS, Konrad TR, Lottenberg R. Blood bank management of sickle cell patients at comprehensive sickle cell centers. Transfusion 2007;47:2089–97.
7 Castro O, Sandler SG, Houston-Yu P, Rana S. Predicting the effect of transfusing only phenotype-matched RBCs to patients with sickle cell disease: theoretical and practical implications. Transfusion 2002;42:684–90.
8 Hoppe C, Klitz W, Vichinsky E, Styles L. HLA type and risk of alloimmunization in sickle cell disease. Am J Hematol 2009;84:462–4.
9. Sakaguchi S, Powrie F. Emerging challenges in regulatory T cell function and biology. Science 2007;317:627–9.
10. Rosse WF, Gallagher D, Kinney TR, et al. Transfusion and alloimmunization in sickle cell disease. The Cooperative Study of Sickle Cell Disease. Blood 1990;76:1431–7.
11. Vichinsky EP, Haberkern CM, Neumayr L, et al. A comparison of conservative and aggressive transfusion regimens in the perioperative management of sickle cell disease. The Preoperative Transfusion in Sickle Cell Disease Study Group [see comments]. N Engl J Med 1995;333:206–13.
12. Vichinsky EP, Luban NL, Wright E, et al. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. Transfusion 2001;41:1086–92.
13. Allen TI, Billingsley K, Slaughter J, et al. Red cell genotyping: a cost effective approach to screening large numbers of donors [abstract]. Transfusion 2009;49(Suppl):135A.
14. Ribeiro KR, Guarnieri MH, da Costa DC, Costa FF, Pellegrino J Jr, Castilho L. DNA array analysis for red blood cell antigens facilitates the transfusion support with antigen-matched blood in patients with sickle cell disease. Vox Sang 2009;97:147–52.
15. Wilkinson K, Harris S, Gaur P, et al. Molecular blood typing augments serologic testing and allows for enhanced matching of red blood cells for transfusion in patients with sickle cell disease. Transfusion 2012;52:381–8.
16. Tournamille C, Meunier-Costes N, Costes B, et al. Partial C antigen in sickle cell disease patients: clinical relevance and prevention of alloimmunization. Transfusion 2010;50:13–19.
17 Young PP, Uzieblo A, Trulock E, Lublin DM, Goodnough LT. Autoantibody formation after alloimmunization: are blood transfusions a risk factor for autoimmune hemolytic anemia? Transfusion 2004;44:67–72.
18. Ballas SK, Marcolina MJ. Hyperhemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anemia. Transfusion 2006;46:105–10.
19. Win N, New H, Lee E, de la Fuente J. Hyperhemolysis syndrome in sickle cell disease: case report (recurrent episode) and literature review. Transfusion 2008;48:1231–8.
20. Bachmeyer C, Maury J, Parrot A, et al. Rituximab as an effective treatment of hyperhemolysis syndrome in sickle cell anemia. Am J Hematol 2010;85:91–2.
21. Ambruso DR, Githens JH, Alcorn R, et al. Experience with donors matched for minor blood group antigens in patients with sickle cell anemia who are receiving chronic transfusion therapy. Transfusion 1987;27:94–8.
22. Lasalle-Williams M, Nuss R, Le T, et al. Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center (CME). Transfusion 2011;51:1732–9.
23. Osby M, Shulman IA. Phenotype matching of donor red blood cell units for nonalloimmunized sickle cell disease patients: a survey of 1182 North American laboratories. Arch Pathol Lab Med 2005;129:190–3.
24. Ambruso DR, Pallas C, Dickey W, et al. Prevention of alloimmunization in sickle cell disease: update on a successful and cost-effective regional program [abstract]. Presented at the 20th Meeting of the National Sickle Cell Disease Program. Boston, MA, March 18–21, 1995.

Elliott Vichinsky, MD, Medical Director, Hematology/Oncology Dept., Children's Hospital and Research Center Oakland, 747 52nd Street, Oakland, CA 94609.