Transfusion practices for patients with sickle cell disease at the Children's Hospital of Philadelphia

S.T. Chou and D.F. Friedman

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The Comprehensive Sickle Cell Center (CSCC) at the Children's Hospital of Philadelphia (CHOP) provides routine and acute care for approximately 1000 patients with sickle cell disease (SCD). One hundred twenty patients with SCD are currently managed with chronic transfusion therapy, either by simple transfusion or by erythrocytapheresis to maintain a percent hemoglobin S (HbS) level of less than 30 or 50 percent, depending on the indication. The most common indication for chronic transfusion therapy at our institution is stroke prevention, either primary or secondary, followed by recurrent acute chest syndrome and splenic sequestration, and, less commonly, chronic cardiac disease or severe recurrent vaso-occlusive episodes. Many children receive acute or episodic red blood cell (RBC) transfusions when admitted to the hospital for parvovirus-associated aplastic crisis, acute chest syndrome, splenic sequestration, stroke, and transient ischemic attacks, or as preoperative therapy to decrease the risk of acute chest syndrome after general anesthesia.

Although transfusion therapy remains a mainstay for the treatment of acute and chronic complications of SCD, the risk of alloimmunization to minor RBC antigens continues to be a significant complication.[1–5] Alloimmunization can have severe clinical consequences, not only because it can lead to significant delays in providing compatible blood, but also because alloimmunization in patients with SCD is associated with delayed hemolytic transfusion reactions (DHTR), autoantibody formation, and hyperhemolysis (reviewed in Wahl and Quirolo[6]). At CHOP, we have implemented specialized transfusion protocols for patients with SCD to help decrease alloimmunization risk.

Transfusion Protocol

More than two thirds of alloantibodies formed by patients with SCD have Rh blood group (D, E, e, C, c) specificities, and the three most common alloantibodies in patients with SCD are directed against C, E, and K.[4,7] Despite efforts to address this problem by prophylactic matching for C, E, and K (in addition to routine matching for ABO and D), about 10 percent of transfused patients with SCD still become alloimmunized.[3,5] Our current practice for transfusion of patients with SCD is to match prospectively for C, E, and K, and to provide ethnically matched RBC units when possible. An extended RBC phenotype is performed on samples from all patients with SCD using an untransfused specimen, typically within the first year of life or at the first outpatient visit if transferring care from another institution. We determine ABO, D, C, c, E, e, K, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S, s, Le^a, Le^b, and P1 status by serologic methods. If a patient has been recently transfused, DNA-based methods are used to determine the predicted RBC phenotype. The extended phenotype is often helpful in guiding the serologic workup of new RBC antibody findings, as well as in prospective matching for additional minor antigens. For patients who have developed multiple alloantibodies or have a history of hyperhemolysis syndrome, we prospectively match RBC units for additional antigens, typically in the Kidd, Duffy, and MNS systems. This extended phenotype matching is performed on an individual case basis, taking into consideration the alloantibodies already formed, the antigens still at risk for antibody development, and the feasibility of the resulting extended match request.

Antibodies to low-incidence RBC antigens are also observed in our chronically transfused patients. Although these antigens are considered low-incidence because they occur in less than 1 percent of the general donor population, some are found at much higher incidence among African American donors.[8] In many cases, reagents to screen RBC units for these antigens are not readily available. The blood supplier may be able to provide units from donors known historically to be antigen negative even if current donations cannot be screened. Two additional strategies are used to screen units for transfusion to patients with antibodies to low-incidence antigens. If the specificity is demonstrable in the

current serum sample based on reactivity with cells known to express the low-incidence antigen, then a negative anti-human globulin (AHG) phase crossmatch is accepted as an adequate screen for antigen-negative donor units. If the specificity is not demonstrable in the current serum sample, we may be able to use confirmed antibody-positive serum samples from the same patient that have been retained in frozen aliquots for this purpose. If none of these measures is available to screen for low-incidence antigens, RBC units must be issued with an unscreened status, for which our policy requires clinician acknowledgment by signature on a protocol waiver.

Matched blood for patients with SCD is most likely to be found among African American donors, whose RBCs more commonly lack C, E, K, S, Fy^a, Fy^b, and Jk^b compared with Caucasian donors.[9] In 1997, CHOP, the Penn-Jersey American Red Cross (ARC), and the local chapter of the Sickle Cell Disease Association of America initiated the Blue-Tag Program to direct blood from African American donors to children with SCD.[10] Blood donors voluntarily self-identify as African American and agree to have their blood specifically support children with SCD by attaching a special blue tag to their donation. With 1200 to 1600 RBC units collected per month, these donors support the majority of transfusions for patients with SCD at CHOP and St. Christopher's Hospital for Children. The primary reason a patient with SCD may not receive program units is the presence of alloantibodies that preclude an appropriate match from the Blue-Tag RBC inventory.

The issue of RBC alloimmunization within the Rh blood group system is greatly complicated by the genetic diversity of this locus within populations of African origin and the limitation of current serologic reagents to distinguish the many variant antigens. Altered D, C, and e often underlie complex Rh alloimmunization in patients with SCD. Many of these cases appear as apparent autoantibodies with relative specificities in the Rh system; however, molecular analysis reveals that many of these antibodies represent alloantibodies in the Rh system for which serologic reagents are not available. *RH* genetic testing can now be used in the clinical setting to detect altered *RHD* and *RHCE* in individuals at risk for producing antibodies to high-incidence Rh antigens.[11] We use genotyping to resolve complex Rh antibodies, particularly for those patients who develop antibodies in the face of conventional antigen matching. Additionally, we obtain an *RH* genotype to supplement the RBC antigen profile for all chronically transfused patients with SCD.

One common variant *RH* allele in African Americans is the hybrid *RHD-CE-D,* which results from *RHCE* exons 3 through 7 replacing the region of *RHD* encoding D epitopes.[12] The RBCs with this hybrid protein type as D– and C+, but patients often develop anti-C when exposed to C+ RBCs, and this can be associated with a DHTR. In our blood bank, patients with the hybrid *RHD-CE-D* who lack an *RHCE* allele encoding conventional C receive C– RBCs to prospectively prevent anti-C production, consistent with the general policy for prophylactic C matching. Additional RBC matching for Rh variants is not performed because the clinical relevance of most variant alleles is unknown.[13] Future studies to determine which additional variants should be included in prospective matching to prevent alloimmunization and hemolytic transfusion reactions is paramount, as molecular testing could provide superior outcomes.

Donor RBC Selection Protocols

In addition to performing prospective C, E, and K antigen matching and providing ethnically matched RBCs when possible, we provide patients with SCD HbS-negative units that are screened by the blood bank via a rapid solubility test. Because the survival of transfused RBCs declines with storage, we provide RBC units collected within 21 days for patients on chronic transfusion programs. The goal is to improve RBC survival, maintain the desired HbS level between transfusion visits, and achieve longer transfusion intervals with concomitantly decreased iron burden. For episodic or acute transfusion of patients with SCD, we do not have a restriction on the age of the RBC units issued. The CHOP blood bank provides prestorage leukocyte-reduced cytomegalovirus (CMV)-safe blood to all patients, including those with SCD. Irradiation is performed on-site, just before issuance for transfusion, and is applied to the great majority of transfusions using protocols based on patient location and service. One exception is RBC units ordered on-call for the operating room for potential bleeding, in which the units are not irradiated unless medically indicated for the patient. In the case of SCD, on-call units would be irradiated. Many of our patients with SCD, particularly those who are chronically transfused, require washed RBC components owing to recurrent allergic or cytokine-mediated transfusion reactions despite premedications. RBC washing is performed on-site and within 24 hours of transfusion.

Emergency Protocols

If emergent transfusion is necessary, the blood bank will issue uncrossmatched O– units or uncrossmatched type-specific blood (based on historical ABO type on at least two

prior specimens performed by the CHOP blood bank). Both protocols require a waiver signed by the treating physician. In addition, if time allows, C-, E-, and K-matched or antigen-negative uncrossmatched units can be requested by the medical team. Because we transfuse a large number of patients with SCD on a chronic basis, units known to be negative for C, E, and K are usually on the shelf and readily available for emergency transfusion. At CHOP, the emergency department and intensive care units have satellite blood component refrigerators that store O– RBC units that are unscreened for C, E, and K. These components have occasionally been used for patients with SCD who have life-threatening anemia requiring emergent transfusion. When uncrossmatched blood is transfused, whether issued by the blood bank or from the satellite blood component refrigerators, a retrospective crossmatch is performed with the patient's specimen and a segment of the RBC unit that is retained in the blood bank. For patients with SCD, we would also retrospectively phenotype unscreened RBC units for their C, E, and K status to provide exposure information to the medical team.

Warm Autoantibodies in Patients With SCD

It has been our experience that many patients with SCD who are chronically transfused form warm autoantibodies detectable for various lengths of time in the antibody screen. If a patient demonstrates a warm autoantibody in the serum, a sample is usually sent to our reference laboratory for allo- or auto-adsorption studies to evaluate for underlying alloantibodies. For chronically transfused patients, we typically arrange for blood samples to be drawn 2 to 3 days before a scheduled transfusion so that these adsorption studies can be performed in advance. In part because of proximity, our reference laboratory can also perform these studies within 6 hours for emergent situations. After the adsorption studies are completed, we request RBC units from our blood supplier that are matched for C, E, K, and any other antigens against which the patient has a past or current history of alloimmunization; crossmatches are performed in our blood bank, and the least incompatible units are issued for transfusion. Currently, we use the gel method for routine antibody screening and crossmatching. If a warm autoantibody is a repetitive finding for a chronically transfused patient, our blood bank may repeat the antibody screen using a low-ionic strength saline tube method, and if the warm autoantibody is not detectable by tube method, we often elect to perform subsequent antibody screens and crossmatches for that particular patient using the tube method.

Protocol Outcomes and Future Directions

More than 5000 RBC units are transfused each year at CHOP to patients with SCD, which constitutes more than 40 percent of all RBC units issued by our blood bank. Selecting and providing the appropriate RBC units requires tremendous coordination between the CHOP blood bank, hematology service, apheresis team, and the Penn-Jersey ARC, which supplies the blood components. The risk of alloimmunization and DHTRs for patients with SCD has been significantly minimized by prospective partial phenotype matching in the Rh and Kell blood group systems. Additionally, the Blue-Tag African American blood donor recruitment program in Philadelphia has increased the local supply of ethnically similar RBCs whose blood group antigen profiles more closely match the patients'. From a cost perspective, 4.7 percent of RBC units issued to patients with SCD were matched for a single antigen, 33 percent were matched for two antigens, and 61 percent were matched for three antigens, with the remaining matched for more than three antigens. This antigen matching increased the blood acquisition cost for the program by 65 percent over the base cost of RBC units. In addition, a handling fee for the Blue-Tag donor program added 3 percent to the overall cost for the program.

Most recently, *RH* genotyping of patients has improved our understanding of Rh antibody production in SCD and offers the possibility of eliminating further Rh alloimmunization if partnered with genotyping of donors. Commercial polymerase chain reaction–based technology is now available to detect the majority of *RH* variants, allowing high-throughput genotyping of donors and patients to select more accurately matched RBCs. Our goal is to characterize *RH* in all patients with SCD, to identify those who would benefit from *RH* genetically matched transfusions, and to improve transfusion outcomes by applying molecular methods in transfusion medicine.

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Stella T. Chou, MD (corresponding author), Assistant Professor of Pediatrics, and David F. Friedman, MD, Clinical Assistant Professor of Pediatrics, The Perelman School of Medicine at the University of Pennsylvania and The Children's Hospital of Philadelphia, Department of Pediatrics, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104.