



How I safely transfuse patients with sickle-cell disease and manage delayed hemolytic transfusion reactions

France Pirenne^{1,2} and Karina Yazdanbakhsh³

¹Etablissement Français du Sang, INSERM U955, Université Paris Est Créteil, Créteil, France; ²Laboratoire d'Excellence GR-Ex, Paris, France; and ³Laboratory of Complement Biology, New York Blood Center, New York, NY

Transfusions can be a life-saving treatment of patients with sickle-cell disease (SCD). However, availability of matched units can be limiting because of distinctive blood group polymorphisms in patients of African descent. Development of antibodies against the transfused red blood cells (RBCs), resulting in delayed hemolytic transfusion reactions (DHTRs), can be life-threatening and pose unique challenges for this population with regard to treatment strategies and transfusion management protocols. In cases where the transfused cells and the patient's own RBCs are destroyed, diagnosis of DHTR can be difficult because symptoms may mimic vaso-occlusive crisis, and frequently, antibodies are undetectable. Guidelines are needed for early diagnosis of DHTR because treatment may need to include temporarily withholding any new transfusions to avoid further hemolysis. Also needed are case-control studies to optimally tailor treatments based on the severity of DHTR and develop preventive transfusion strategies for patients at DHTR risk. Here, we will review gaps in knowledge and describe through case studies our recommended approach to prevent alloimmunization and to diagnose and treat symptomatic DHTRs for which complementary mechanistic studies to understand their pathogenesis are sorely needed. (*Blood*. 2018;131(25):2773-2781)

Introduction

Transfusions can be life-saving for patients with sickle-cell disease (SCD),¹⁻³ but patients may develop antibodies against transfused red blood cells (RBCs) resulting in a delayed hemolytic transfusion reaction (DHTR). DHTRs are classically caused by an anamnestic reaction where alloantibodies undetectable at the time of transfusion rebound following exposure to the corresponding RBC antigen. Antibody-coated donor RBCs, thus detected by a positive direct antiglobulin test (DAT), are destroyed by immune effector cells and/or complement activation. However, in many cases of SCD DHTR, no alloantibodies are detected.⁴ In addition, in the severest cases, hyperhemolysis defined by the destruction of both transfused and autologous RBCs occurs⁵⁻⁹; this may be accompanied by reticulocytopenia, worsening the anemia. Hyperhemolysis can induce multiorgan failure (MOF) and death, most likely because of damage to the underlying vasculature by released free hemoglobin (Hb) and heme.^{10,11} Additional transfusions are frequently prescribed but can worsen hemolysis. Based on retrospective studies, the incidence of severe DHTR is 11.5% to 16% with only ABO and RhD matching and 4% to 7% in patients matched for Rh (D, C, E, c, e) and K (supplemental Table 1, available on the *Blood* Web site); in an adult cohort, DHTRs represented 4.2% of all causes of death in SCD.¹² These severity and mortality rates likely underestimate true incidences because DHTRs are frequently misdiagnosed as simple vaso-occlusive episodes (VOEs).

A salient feature of DHTR in SCD is that its occurrence, as well as its clinical progression from mild to severe, is unpredictable. This

underscores the need for careful monitoring of transfusion outcomes in SCD to ensure correct diagnosis and appropriate treatment, including temporarily withholding additional transfusions to avoid further hemolysis. Prevention of DHTR in SCD is challenging, partly because little is known regarding mechanisms of DHTRs in which no antibodies are detectable.⁴ Here, we present 3 cases that highlight approaches for prevention, diagnosis, and treatment of DHTR, in a field in which evidence-based studies are sorely lacking.

Prevention of DHTR

Case 1

A 35-year-old woman presented for cardiac surgery with a history of 2 prior DHTRs at age 30 and 32. Her blood type is D+ C-E-c+e+, K-, Fy(a-b-), Jk(a+b-), S-s+. Prior to the 2 DHTRs, she had previously developed anti-C, K, and auto-anti-e. At age 30, DHTR was triggered by development of anti-S and anti-Lu^a with prophylactic transfusion during pregnancy of crossmatch-compatible RBCs (C-E-k-Fy^a-Jk^b-). At age 32, DHTR was triggered by transfusion for abortion with development of anti-E despite provision of E-negative and crossmatch-compatible RBCs (C-E-k-Fy^a-Jk^b-S-). In both instances, she was admitted to the intensive care unit (ICU) with severe pain, hemoglobinuria, and evidence of hemolysis, with an lactate dehydrogenase (LDH) increase from 320 to 1550 and 2058 IU/L, respectively. Her Hb dropped 2 g/dL between days 2 and 10 after transfusion in the first DHTR, and 3 g/dL between days 2 and 12 in the second DHTR.

For the cardiac surgery, she received 2 1000-mg doses of rituximab prophylaxis at 1 month and 15 days before surgery. She received 2 units of crossmatch-compatible RBCs (C–E–k–Fy^a–Jk^b–S–), with a day 1 posttransfusion Hb of 7.7 g/dL and HbA% of 62. Her clinical course was uneventful, with Hb 8.6 g/dL and HbA% 37 at day 25 and no new antibodies in follow-up (3 months). The patient had previously received antipneumococcal vaccine and received prophylactic antibiotics for 3 months.

This high-DHTR-risk case illustrates how an immunization prevention strategy likely prevented development of DHTR.

How to prevent alloimmunization?

What do we know about alloimmunization in SCD? The incidence of alloimmunization is high in SCD,¹³ in part because of higher prevalence of C, E, Fy^a, Jk^b, and S polymorphic blood group antigens in donors of primarily European descent than patients of African origin.^{14–18} In addition, transfusion burden and exposure, as measured by cumulative number of transfusions, is higher in SCD and linearly correlates with SCD alloimmunization.¹⁹

The higher alloimmunization in SCD may also be because of the inflammatory nature of this condition. Studies in experimental models, although not seen in mouse SCD models,²⁰ indicate that recipient inflammatory state increases the risk of alloimmunization. In a retrospective study, SCD patients transfused for acute, and therefore inflamed, complications had increased alloimmunization risk.²¹

Some patients, so-called responders, become alloimmunized early during transfusion therapy, whereas others never become immunized, invoking the concept of genetic predisposition to alloimmunization.¹⁹ Genetic studies have also identified gene polymorphisms encoding immune modulatory proteins including TRIM21, CD81, and CTLA-4, as well as certain HLA alleles associated with SCD alloimmunization.^{22–25} A genetic variant in the Fcγ receptor gene was recently associated with decreased risk of SCD alloimmunization, although interestingly, this variant did not protect against alloimmunization to the highly immunogenic Rh and K antigens.²⁶ Once validated in larger cohorts, such genetic markers may help risk stratify SCD patients, ensuring that use of extended and genotype-matched units are reserved for patients at highest risk of alloimmunization. Functional immune studies comparing alloantibody responders with nonresponders have unraveled aberrant molecular pathways potentially associated with alloimmunization, including innate immune response to heme, follicular helper T-cell subset signaling, and regulatory T-cell suppressive pathways.^{27–33} Prospective studies, following patients with SCD as they become alloimmunized, are needed to determine whether these immunological alterations are the cause or effect of alloimmunization. Although the latter may help identify novel targeted therapies to reverse or prevent alloimmunization, the former is essential for identifying alloimmunization risk biomarkers.

Antigen matching: what and how much to match? Transfusing SCD patients with RBCs matched for Rh (D, C, E, c, e) and K antigens is the standard of care in many centers.^{34–36} Individuals of African descent have a high degree of genetic variation in the *RH* locus,^{37,38} and thus may have *RH* variants encoding so-called partial Rh antigens, which lack certain immunogenic epitopes of the normal antigen. Such patients, even when

receiving Rh serologic-phenotype matched units from minority donors, may develop anti-Rh antibodies against those missing epitopes when transfused with RBCs carrying the “normal” antigen (see supplemental Figure 1 for examples of partial D antigens). In a pediatric population receiving Rh (D, C, E, c, e) and K matched units, 45% of the chronically and 12% of the episodically transfused recipients still became Rh alloimmunized,³⁷ with similar results in a patient cohort in France.³⁸ In SCD patients, many partial D, C, and e antigens are described, and importantly, the resulting antibodies are associated with DHTR cases.^{37,39,40} Such alloimmunized patients should be transfused with antigen-negative units (D-negative RBCs for a partial D patient). Although serologic tools can identify some Rh variant antigens, molecular techniques are more specific in identifying and distinguishing between *RH* alleles encoding partial and other variant antigens.⁴¹ However, until costs decrease, we propose that *RH* genotyping to identify partial Rh antigens be performed only in patients already immunized with clinically significant alloantibodies and/or autoantibodies whom we consider high antibody responders, and in patients who develop an Rh antibody despite conventional Rh matching. We also propose that Rh partial antigen matching as an alloimmunization prevention strategy should be based on antigen-negative unit availability and reserved for the high responders because not all patients with Rh variants become alloimmunized.

Extended matching to Fy, Jk, and Ss blood groups can further reduce alloimmunization³⁶ but is not standard practice, in part because of insufficient supply of extended matched units for managing the routine transfusion needs of all patients with SCD. Furthermore, alloimmunization against Fy, Jk, and Ss occurs in only 5% to 15% of polytransfused patients.^{21,37,38} Therefore, extended matched units should be reserved only for patients in need, including those who develop the corresponding alloantibodies.

Why the need for an additional preventive strategy against RBC immunization? Although detectable alloantibodies in SCD DHTRs are frequently against antigens such as Rh, K, Fy, Jk, and Ss, patients can also develop antibodies against many other RBC antigens, including low-frequency antigens as well as autoantibodies and nonspecific antibodies.^{15,42} Although clinical relevance of some of these antibodies in DHTR is not completely understood, severe DHTRs associated with autoantibodies or low-frequency alloantibodies have been reported.^{43,44} B-cell depletion therapy has therefore been empirically used to prevent RBC immunization in high-risk patients (those already immunized and with history of DHTR). In many cases, prophylactic rituximab has prevented alloimmunization and DHTRs.^{43,45,46} In 1 case study, however, its use was associated with fatal outcome,⁴⁷ and in a case series involving 8 patients, all with favorable outcomes, mild DHTR still developed in 3 patients.⁴⁵ Although case control studies are needed to more definitively establish efficacy, rituximab can be considered when a new transfusion is absolutely necessary in patients with a history of severe DHTR and evidence of being a high antibody responder. Patients receiving rituximab require antipneumococcal vaccination, which is already recommended for asplenic SCD patients, and antibiotics (twice a day, 1 MU penicillin V or 500 mg penicillin) until CD19⁺ B-cell counts recover. Corticosteroid is also typically administered with rituximab for prevention of hypersensitivity reactions; only a low dose (methylprednisolone, 10 mg) should be given to SCD patients, to avoid corticosteroid-induced VOE.

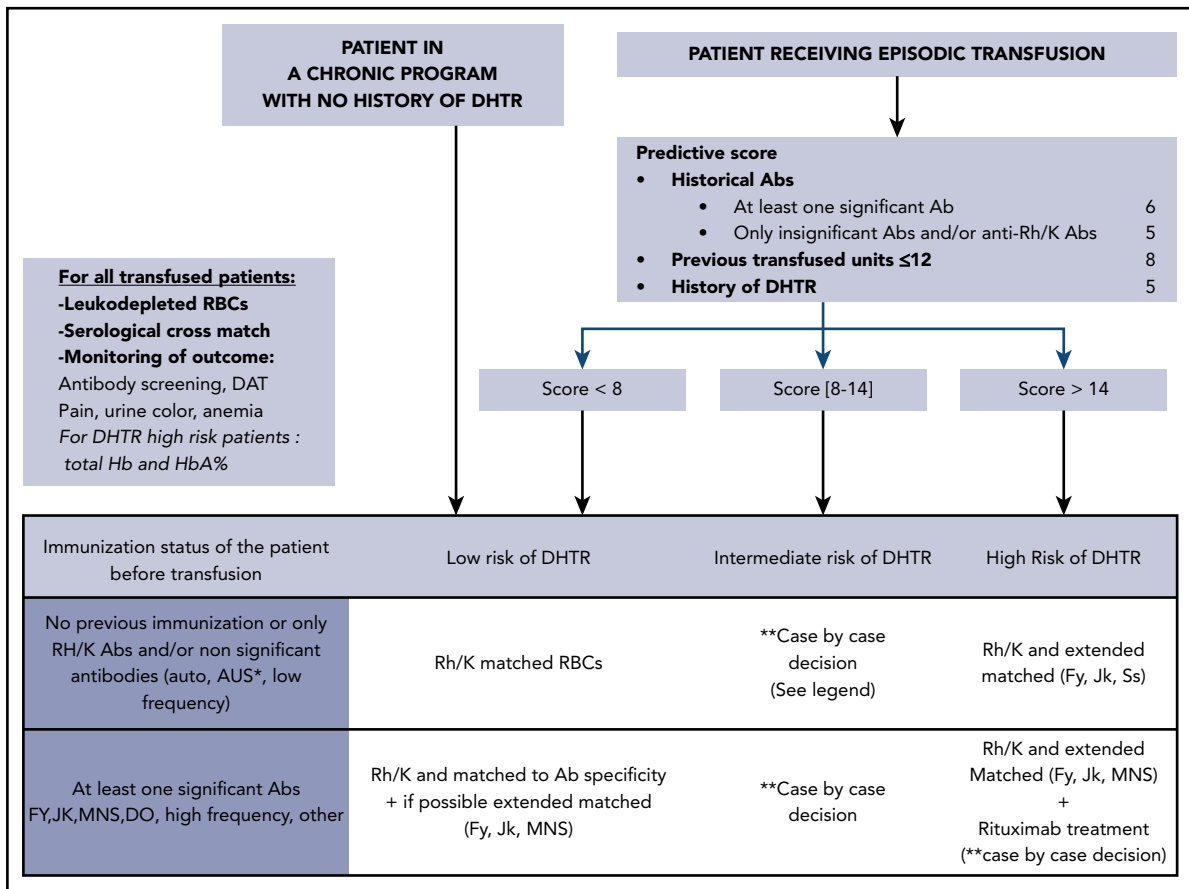


Figure 1. Recommended transfusion guidelines for patients with SCD. All transfused patients with SCD should receive serologically crossmatch-compatible leukodepleted RBCs and be monitored carefully by performing regular antibody screening tests including DAT and checking for pain, urine color, and signs of anemia. Total Hb and HbA% measurements (Figure 2) are recommended for patients at high risk of DHTR. Patients on a chronic transfusion protocol are considered low-risk DHTR. For patients receiving episodic transfusions, 3 criteria, assigned different point values based on statistical analysis,⁴⁸ are considered DHTR risk factors: (1) History of RBC immunization. A point value of 6 is given if the patient has a history of at least 1 clinically significant antibody (other than anti-Rh or anti-K) classically known to be involved in transfusion reactions, such as anti-Jk^b, Fy^a, S, Hr^s. A point value of 5 is given if the patient has a history of only anti-Rh/-K and/or antibodies considered not clinically (clin) significant (eg, autoantibodies or nonspecific antibodies [Ab's]). Thus, a patient who has an anti-Rh plus an anti-Jk^b is given a point value of 6 (and not 6 + 5). (2) Cumulative transfusions of 12 units or less. (3) A previous DHTR. By adding the point values, a DHTR risk score is calculated and transfusion is tailored accordingly. Patients with a score of <8 are considered low risk. Patients transfused episodically who have a low risk of DHTR are transfused with Rh (D, C, E, c, e) and K matched RBCs, which is extended to Fy, Jk, and Ss only if the patient has developed antibodies against any one of these antigens. *AUS, antibody with unknown specificity. **Patients who score between 8 and 14 have an intermediate risk. For such patients, the extent of matching should be based on their DHTR history and number of previous transfusions; those with no history of DHTR who have been transfused only a few times are considered at a lower risk similar to low-risk patients, but they should still be monitored closely. However, for patients with intermediate DHTR risk who have a history of DHTRs and few transfusions in the past (≤12), we generally consider them high risk, and they receive extended matched RBCs (Fy, Jk, Ss). Patients with a score of >14 are considered at high DHTR risk. Episodically transfused patients with a high risk of DHTR (based on the predictive score) always receive extended matched RBCs (Fy, Jk, Ss). Prophylactic rituximab use should be considered for patients with a history of alloantibodies and severe DHTR.

Beyond limiting development of new RBC antibodies, rituximab prophylaxis is not indicated in prevention of alloantibody-negative DHTRs.

Proposed DHTR prevention strategies based on known risks of DHTR

Given that DHTRs are unpredictable and often life threatening, identifying risk factors by patient history and presentation is urgently needed in order to risk stratify the transfusion regimen.

Many studies suggest that 3 factors increase the risk of DHTR: (1) history of immunization, (2) previous history of DHTRs, and (3) transfusion for an acute complication. A lower cumulative number of transfused units (≤12 units) may also be a risk factor in adult patients.⁴⁸ Odds ratios from a single-institution multivariate

analysis of DHTR risks were used to create a scoring system to predict the probability of DHTR and accordingly adapt transfusion protocols (Figure 1).⁴⁸

Patients with no history of DHTR on chronic transfusion have a lower risk of developing DHTR. In fact, in a recent prospective study of adults, DHTR was demonstrated exclusively in patients receiving episodic transfusions.⁴⁸ Within that group, history of RBC immunization and a previous DHTR dramatically increased DHTR risk. These 3 risk factors (number of previous transfusions, history of immunization, and previous DHTRs) should thus be carefully considered when evaluating a patient for transfusion. If transfusion is necessary in high-risk patients, preventing alloimmunization should be prioritized because it may induce DHTR. For patients with anti-Fy, -Jk, or -Ss, we recommend extended matching (Fy, Jk, Ss) because the risk of producing

additional antibodies increases with each new transfusion.^{19,38} Given their high likelihood of alloimmunization against any blood group antigen, we also recommend prophylaxis with rituximab prior to transfusion in this patient group.

Prevention of alloantibody-negative DHTR is challenging. Although there currently is no known DHTR preventive measure for this type of patient, one must rule out the possibility of actual involvement of antibodies, such as those against low-frequency antigens that require the use of specific RBCs for their detection or evanescent antibodies.⁴⁹ Follow-up antibody screens at set intervals will maximize detection of any possible posttransfusion antibodies, which once identified, help selection of units for future transfusions to avoid restimulation. For patients who are nonalloimmunized but have a history of DHTR and few previous transfusions, we propose empiric extended matching (Fy, Jk, Ss) alone. Rituximab prophylaxis is not indicated unless antibody production was possibly missed, as described previously. The mechanism(s) that trigger RBC destruction in confirmed cases of antibody-negative DHTR are largely unknown, but possible mechanisms, which remain to be demonstrated, could involve heme-driven alternative complement activation pathways, suicidal death of transfused aged-stored RBCs, or destruction of G6PD-deficient RBCs.⁵⁰⁻⁵² Mechanistic insights to understand this clinical enigma may help guide development of optimal prevention strategies for such cases.

In low-risk patients, such as those heavily transfused with no DHTR history, or episodically transfused with a low risk score (Figure 1), matching for the highly immunogenic Rh (D, C, E, c, e) and K antigens should be the standard of care. The risk of alloimmunization to Rh variants, however, remains high in all patients,^{18,19} and the decision to match for these variants needs to be determined on a case-by-case basis. We consider unnecessary, however, prophylactic extended matching (Fy, Jk, Ss) in patients who have antibodies against only Rh (D, C, E, c, e) and K, and no other DHTR risk. The scoring system also provides recommendations for prophylactic matching for patients with intermediate risk based on DHTR history and number of previous transfusions.

This is the first published stratification system for DHTR risk and prevention and thus needs to be validated by other institutions and in different patient cohorts including SCD pediatric patients. It can be implemented only if the patient transfusion history is known, a not-so-trivial hurdle because patients may receive transfusions at multiple hospitals. This prevention strategy based on the above DHTR risk criteria has been successfully implemented at a single French facility, with ongoing efforts to expand it nationally.

Diagnosis and treatment of DHTR

Case 2

A 26-year-old woman with SCD was admitted to the ICU for severe acute chest syndrome with pulmonary hypertension. She had previously received multiple episodic transfusions (56 units) but never experienced DHTR and had no previous immunization history. Pretransfusion antibody screening was negative. The patient typed as D–C–E–c+e+, K–, Fy(a–b–), Jk(a+b+), S+s+. On day 0, she received a partial manual exchange of 2 units of

crossmatch-compatible RBCs (D–C–E–K–). After a second partial manual exchange on day 3, the total Hb was 10 g/dL; HbA%, 43.6; and LDH, 300 IU/L. Her condition improved, and she was transferred out of the ICU. On day 7, however, she developed severe pain and dark urine with Hb of 6.6 g/dL, HbA % of 29, LDH of 3082 IU/L, and total bilirubin of 64 mmol/L. She was readmitted to the ICU with the diagnosis of DHTR (Figure 2B). The DAT was positive with immunoglobulin G, but no antibody was identified. She was initially treated with IVIg over 4 days. With further deterioration of her respiratory parameters, recurrence of pulmonary hypertension, and a rapid drop of total Hb, she also received eculizumab 900 mg on day 8 and day 14. Because of life-threatening anemia (2 g/dL), she received 1 unit of extended-matched RBCs. Rituximab was also given because of the positive DAT. At day 15, the Hb stabilized at 3 g/dL and progressively corrected with EPO treatment. Prophylactic anticoagulation was administered to lower the risk of thrombosis associated with EPO administration and decreased mobility in the ICU. The patient improved and was finally discharged from the ICU.

Case 2 highlights the difficulty in predicting the occurrence of DHTR (Figure 1), underscoring that low-risk patients are not risk free and the need for better mechanistic understanding of DHTR. This case also demonstrates a strategy for diagnosis and a possible treatment course for hyperhemolytic cases.

Diagnosis of DHTR

Currently, there is no consensus definition of DHTR. We use DHTR as a broad term that encompasses cases without detectable antibodies, but with unequivocal evidence of marked hemolysis within a given time frame, typically a few hours to 3 weeks after transfusion.^{9,53} In adults, we recommend a 2-step process for diagnosis of DHTR (Figure 2A), based on published case report descriptions and the change in HbA concentration. The first step is recognizing the following: recurrence or appearance of VOE following a recent transfusion, dark urine, onset or worsening of anemia, and increased LDH. These features should alert professionals and patients alike to probable DHTR. This first step recognition is crucial to prevent further transfusions exacerbating hemolysis and to initiate treatment before irreversible multiple organ failure. The second step is determining the extent of HbA concentration drop relative to the immediate posttransfusion values. We have designed in adults a nomogram for the confirmation of ongoing DHTR⁵⁴ (Figure 2A), which relies on assessment of immediate posttransfusion total Hb and HbA% and is recommended at least for patients at high risk of DHTR (Figure 1). Using this algorithm, we made an early diagnosis of DHTR in case 2 (Figure 2B) and also confirmed that DHTR did not develop in the high-risk case 1. This algorithm has to be validated in different patient cohorts and institutions in order to establish the costs (monetary) vs benefits of (reduced morbidity and mortality) of such measurements. In a recent study in 1 institution in children, HbA clearance was also calculated requiring only the volume of RBCs transfused and the hematocrit of the units.⁵² As HbA% measurements may be unavailable, a drop of $\geq 25\%$ in total Hb from the pretransfusion level should raise suspicion for DHTR.⁵³ Future studies are needed to compare the robustness of the various Hb measurements for DHTR diagnosis.

Finally, for DHTR cases where no antibodies are detected, we recommend antibody screening be repeated at regular intervals

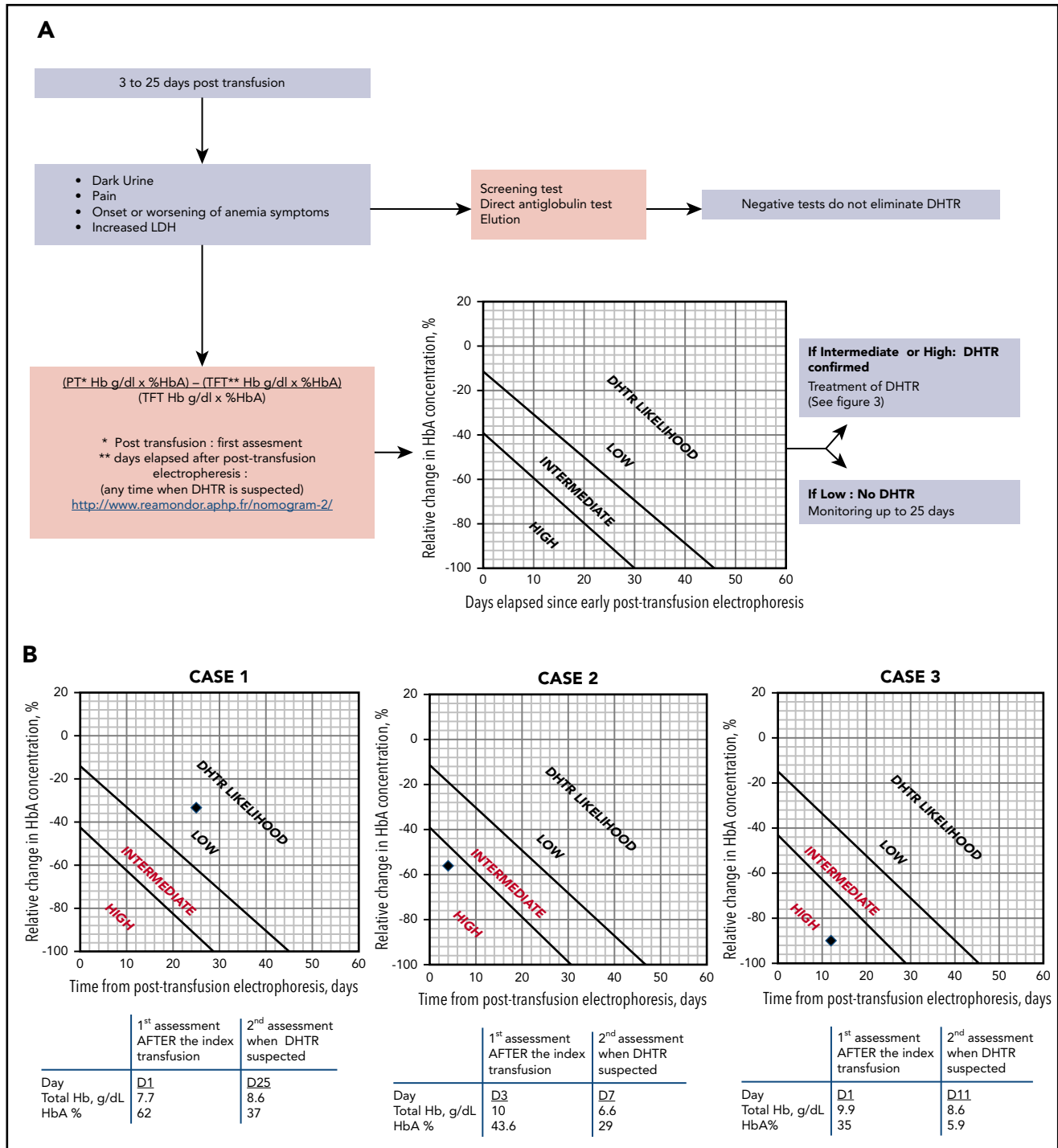


Figure 2. Recommended guidelines for diagnosis of DHTR in adult patients with SCD. (A) Our assessment criteria to diagnose DHTR in adult SCD patients who are recently transfused is based on clinical and laboratory features (pain, anemia, urine color, elevated LDH) and immune-hematologic analysis, which may or may not reveal the presence of new antibodies. If DHTR is suspected, we recommend using immediate posttransfusion total Hb in g/dL and HbA% following the nomogram shown, to determine the likelihood that a patient is experiencing DHTR. The formula can be calculated directly by using the link provided in the figure. (B) Based on total Hb and HbA%, DHTR was suspected for cases 2 and 3, but not for case 1, as confirmed by the nomogram.

within 3 months from the diagnosis of DHTR for detection of de novo antibodies.

Treatment

Many questions remain regarding treatment of DHTR: Is tailored treatment necessary? When can a patient safely receive additional

transfusions? There is little evidence available, and therefore, expertise guides the current recommendations proposed in Figure 3. First, continuous monitoring of all SCD patients experiencing symptomatic DHTR is critical because there are few indicators for predicting progression to life-threatening severity. Along with supportive treatment, a decision algorithm for

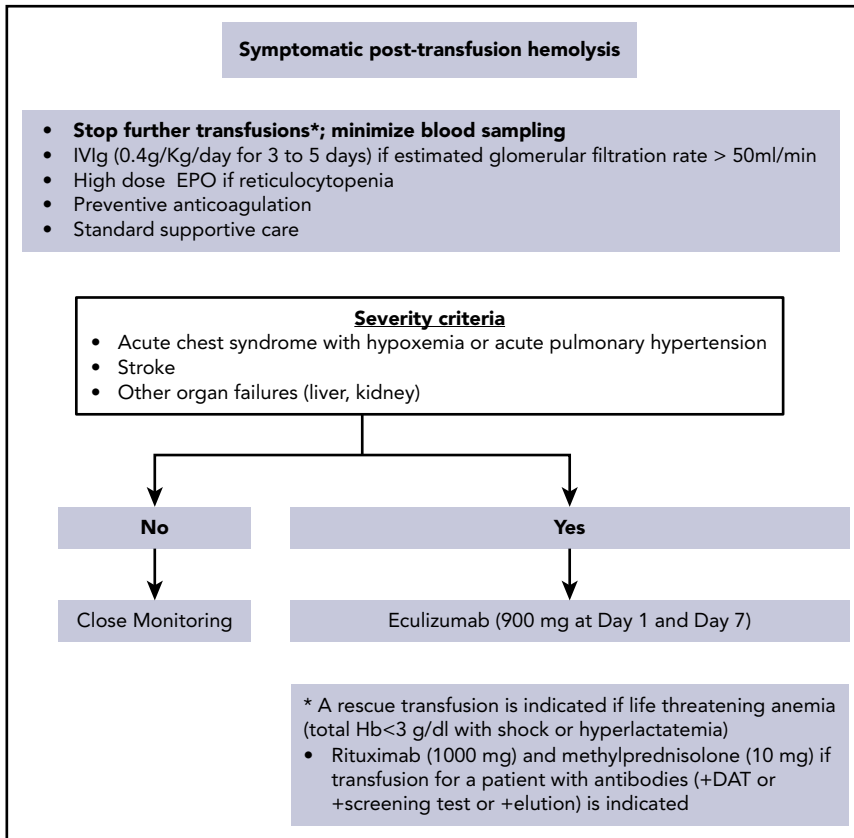


Figure 3. Recommended treatment of patients with SCD experiencing posttransfusion hemolysis. In such cases, transfusions should be stopped unless (indicated by asterisk) the patient has profound anemia (total <3 g/dL with shock or hyperlactemia), in which case rituximab is also indicated for patients with antibodies requiring transfusions. Symptomatic patients experiencing DHTR can be immediately treated with intravenous immunoglobulin (IVIg), adding erythropoietin (EPO) if the DHTR is also associated with reticulocytopenia. Prophylactic anticoagulation is administered to lower the risk of thrombosis associated with EPO administration. Supportive care is always indicated. If the patient has severity criteria (acute chest syndrome or acute pulmonary hypertension, stroke or organ failures), additional treatment with eculizumab can be effective.

selecting specific treatments, based on DHTR severity criteria, can be used (Figure 3).

EPO All patients with reticulocytopenia should receive EPO to stimulate RBC production. Hydroxyurea should be held until cytopenia resolves. When reticulocyte count is below 200 000/mm³ and Hb < 6 g/dL, we recommend darbepoietin- α at a dose of 100 to 300 μ g (every 48 hours) or epoietin- α at 30 000 U (every other day) until reticulocyte counts increase.

Immunosuppressive treatment IVIg therapy is often used for prevention of antibody-mediated immune destruction, although its mechanism of action remains incompletely understood. Despite risks of hyperviscosity, hypercoagulability, kidney toxicity, and even hemolysis in non-blood group O patients, many SCD patients with DHTR have been successfully treated with IVIg.^{55,56} With very low Hb levels as seen in DHTR, the risk of hyperviscosity decreases. Indeed, we recommend IVIg as a first-line therapy for DHTR patients with symptomatic posttransfusion hemolysis (Figure 3), including patients with no detectable antibodies, because antibodies may develop at a later point.

High-dose steroids have been used for immune system modulation, and even as first-line therapy for severe DHTR cases⁹ with or without IVIg; synergy has been reported in suppressing macrophage activity. Steroid use, however, should be weighed against the risk of VOE aggravation. Currently, no guidelines exist for using steroids or IVIg as a first-line treatment, and no evidence-based studies to support the use of one over the other.

For patients with severe DHTR criteria (Figure 3), such as case 2, because the complement cascade can be fully activated,⁵⁷ we recommend salvage therapy with eculizumab, an inhibitor of the C5-convertase, to be administered at the very start of hyperhemolysis to prevent irreversible MOF. Antimeningococcal vaccination is mandatory with eculizumab treatment. Sometimes, as in case 2, the total Hb may continue to drop slowly, despite eculizumab treatment, likely because of delay in erythropoietic response to EPO and other DHTR-associated RBC destruction mechanisms such as erythrophagocytosis.

The type of immune activation in severe DHTRs may differ, however, and patient response to IVIg, steroids, anti-B-cell, and/or anticomplement therapy may likewise differ. In order to tailor treatment, mechanistic studies to understand variable pathophysiology and establishment of alloimmunization and DHTR registries to compare disease presentation and outcomes are badly needed.

Plasma exchange Because free heme and free Hb can have deleterious effects on vasculature,^{10,11} plasma exchange may be effective in severe cases of hyperhemolysis⁵⁸ and was successfully shown to detoxify heme in patients with acute MOF without DHTR.⁵⁹ However, the extracorporeal volume, which can further lower the Hb level, needs to be considered.

Additional transfusions With profound anemia and organ hypoxia, further transfusions may be unavoidable. If transfusion is indicated, as in case 2, we recommend extended matching (Fy, Jk, Ss) in addition to rituximab prophylaxis for patients with existing antibodies. However, because there may be a delay in the appearance of alloantibodies, rituximab can also be given

prophylactically for patients requiring transfusions even when no antibodies are detectable at diagnosis.

Other therapies Use of haptoglobin or hemopexin to detoxify plasma free Hb and free heme, respectively, are potential therapeutic options that might be explored in the future for treatment of DHTR.^{60,61}

SCD transfusion and rare blood type: a difficult challenge

Case 3

A 30-year-old man of sub-Saharan origin was admitted to the ICU for severe acute chest syndrome. In the past, he had received 2 Rh- and K-matched transfusions (4 units). The patient's phenotype was D+C+E-e+c-, K-, Fy(a-b-), Jk(a+b+), S-s+. Following 4 units of crossmatch-compatible RBCs lacking E, c, and K antigens over 2 days, his posttransfusion Hb was 9.9 g/dL, and HbA 35%; with good recovery, he was discharged. However, 10 days posttransfusion, the patient developed a new VOE with Hb 8.6 g/dL, HbA% 5.9, and LDH 1614 IU/L. Diagnosis of DHTR was made (Figure 2B), and the next day his Hb was 4.9 g/dL, and HbA% 0. The patient was monitored closely, and there were no signs of clinical severity despite complete destruction of transfused RBCs. An anti-RH46, associated with a rare Rh blood group (RN), was identified. With EPO and supportive therapy, his Hb increased progressively, and he was discharged 21 days after transfusion.

Rare blood groups: a challenging situation Rare blood groups are mainly defined by lack of expression of high-incidence antigens in any of the 36 known blood group systems.⁶² Certain rare blood types present in individuals of African descent are absent in the primarily white donor population: Hr^s negative, Hr^B negative, and RN in the Rh blood group, U negative in the MNS blood group, and Js^b negative in the KEL blood group. Transfusion and DHTR management in SCD patients with rare blood groups and associated alloimmunization is particularly challenging because rare blood units are in short supply.

Like with partial Rh antigens, all rare blood groups can be identified by molecular techniques, but currently, screening is cost prohibitive. The need for transfusion should be carefully evaluated in patients with rare blood types but not yet alloimmunized. If transfusion is necessary and the patient's responder status is unknown, units with the closest phenotype to the patient's RBCs can be transfused, and the patient closely monitored. However, if the patient is a low responder, transfusions can be given without matching for the rare blood type.

Once a rare blood type patient is alloimmunized, future transfusion needs must be considered. Options include use of cryopreserved rare blood, although this only supports occasional transfusions because of very limited supply even worldwide. More donors of African descent need screening to increase the rare blood type inventory.

Alternative to transfusion in transfusion deadlock: hematopoietic stem cell transplantation (HSCT)

Transfusion deadlock may arise for alloimmunized patients with rare blood types or with multiple alloantibodies. If units are

unavailable for long-term transfusion management, alternative treatments must be considered such as hydroxyurea or HSCT.⁶³ Patients undergoing HSCT require transfusion support, however, so it is critical to secure a supply of matched RBCs, including cryopreserved units if necessary. Even stable mixed chimerism after nonmyeloablative transplant carries a risk of immunohematologic complications. A recent report demonstrated new antibodies against donor or recipient RBCs causing near-fatal hemolysis.⁶⁴ Fortunately, there was no correlation between immunohematologic complications and graft failure, graft rejection, or death. Close consultations between transfusion and transplantation specialists are needed to safely manage transfusion support throughout the transplant.⁶⁵ Gene therapy trials in SCD now underway also hold promise for curing the disease.⁶⁶

Conclusion

Given the challenges of transfusion complications in SCD, a patient blood management plan needs to include strategies to minimize alloimmunization and transfusion protocols tailored by risk stratification of DHTRs; both are contingent on availability of matched units and knowledge of patient transfusion history. Transfusion decisions should be continuously reevaluated, especially for DHTR high-risk patients. Case-control studies for DHTR prevention, diagnosis, and treatment strategies are sorely lacking, highlighting the need to develop nationally shared alloimmunization and DHTR registries to guide future evidence-based studies. Better mechanistic insight into DHTR triggers and pathologic consequences, especially hyperhemolysis, will enable implementation of optimal treatments for this life-threatening condition.

Acknowledgments

The authors thank Anoosha Habibi, Pablo Bartolucci, and Armand Mekontso Dessap for their highly valuable input and discussions, and Patricia Shi for her helpful comments.

This work was supported in part by grants from the National Heart, Lung, and Blood Institute, National Institutes of Health (R01HL121415 and R01HL130139) (K.Y.), and in part by grants from the Etablissement Français du Sang and the Agence Nationale de la Recherche.

Authorship

Contribution: F.P. and K.Y. conceived the manuscript; F.P. drafted the case studies and wrote the first draft of the manuscript; and F.P. and K.Y. edited the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: France Pirenne, Etablissement Français du Sang, INSERM U955, Université Paris Est Créteil, 94000 Créteil, France; e-mail: france.pirenne@efs.sante.fr; and Karina Yazdanbakhsh, Laboratory of Complement Biology, New York Blood Center, 310 East 67th St, New York, NY 10065; e-mail: kyazdanbakhsh@nybc.org.

Footnotes

Submitted 31 January 2018; accepted 1 May 2018. Prepublished online as *Blood* First Edition paper, 3 May 2018; DOI 10.1182/blood-2018-02-785964.

The online version of this article contains a data supplement.

REFERENCES

- 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(1):5-11.
- Cohen AR, Martin MB, Silber JH, Kim HC, Ohene-Frempong K, Schwartz E. A modified transfusion program for prevention of stroke in sickle cell disease. *Blood*. 1992;79(7):1657-1661.
- Oteng-Ntim E, Meeks D, Seed PT, et al. Adverse maternal and perinatal outcomes in pregnant women with sickle cell disease: systematic review and meta-analysis. *Blood*. 2015;125(21):3316-3325.
- Petz LD, Calhoun L, Shulman IA, Johnson C, Herron RM. The sickle cell hemolytic transfusion reaction syndrome. *Transfusion*. 1997;37(4):382-392.
- Habibi A, Mekontso-Dessap A, Guillaud C, et al. Delayed hemolytic transfusion reaction in adult sickle-cell disease: presentations, outcomes, and treatments of 99 referral center episodes. *Am J Hematol*. 2016;91(10):989-994.
- Talano JA, Hillery CA, Gottschall JL, Baylerian DM, Scott JP. Delayed hemolytic transfusion reaction/hyperhemolysis syndrome in children with sickle cell disease. *Pediatrics*. 2003;111(6):e661-e665.
- King KE, Shirey RS, Lankiewicz MW, Young-Ramsaran J, Ness PM. Delayed hemolytic transfusion reactions in sickle cell disease: simultaneous destruction of recipients' red cells. *Transfusion*. 1997;37(4):376-381.
- 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(6):1231-1238.
- Vidler JB, Gardner K, Amenyah K, Mijovic A, Thein SL. Delayed haemolytic transfusion reaction in adults with sickle cell disease: a 5-year experience. *Br J Haematol*. 2015;169(5):746-753.
- Adisa OA, Hu Y, Ghosh S, Aryee D, Osunkwo I, Ofori-Acquah SF. Association between plasma free haem and incidence of vaso-occlusive episodes and acute chest syndrome in children with sickle cell disease. *Br J Haematol*. 2013;162(5):702-705.
- Liu Y, Jing F, Yi W, et al. HO-1^{hi} patrolling monocytes protect against vaso-occlusion in sickle cell disease. *Blood*. 2018;131(14):1600-1610.
- Ngo S, Bartolucci P, Lobo D, et al. Causes of death in sickle cell disease adult patients: old and new trends [abstract]. *Blood*. 2014;124(21). Abstract 2715.
- Rosse WF, Gallagher D, Kinney TR, et al; The Cooperative Study of Sickle Cell Disease. Transfusion and alloimmunization in sickle cell disease. *Blood*. 1990;76(7):1431-1437.
- Vichinsky EP, Earles A, Johnson RA, Hoag MS, Williams A, Lubin B. Alloimmunization in sickle cell anemia and transfusion of racially unmatched blood. *N Engl J Med*. 1990;322(23):1617-1621.
- Aygun B, Padmanabhan S, Paley C, Chandrasekaran V. Clinical significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusions. *Transfusion*. 2002;42(1):37-43.
- Natukunda B, Schonewille H, Ndugwa C, Brand A. Red blood cell alloimmunization in sickle cell disease patients in Uganda. *Transfusion*. 2010;50(1):20-25.
- Godfrey GJ, Lockwood W, Kong M, Bertolone S, Raj A. Antibody development in pediatric sickle cell patients undergoing erythrocytapheresis. *Pediatr Blood Cancer*. 2010;55(6):1134-1137.
- Chou ST, Liem RI, Thompson AA. Challenges of alloimmunization in patients with haemoglobinopathies. *Br J Haematol*. 2012;159(4):394-404.
- Sins JWR, Biemond BJ, van den Berselaar SM, et al. Early occurrence of red blood cell alloimmunization in patients with sickle cell disease. *Am J Hematol*. 2016;91(8):763-769.
- Hendrickson JE, Hod EA, Perry JR, et al. Alloimmunization to transfused HOD red blood cells is not increased in mice with sickle cell disease. *Transfusion*. 2012;52(2):231-240.
- Fasano RM, Booth GS, Miles M, et al. Red blood cell alloimmunization is influenced by recipient inflammatory state at time of transfusion in patients with sickle cell disease. *Br J Haematol*. 2015;168(2):291-300.
- Tatari-Calderone Z, Minniti CP, Kratovil T, et al. rs660 polymorphism in Ro52 (SSA1; TRIM21) is a marker for age-dependent tolerance induction and efficiency of alloimmunization in sickle cell disease. *Mol Immunol*. 2009;47(1):64-70.
- Schonewille H, Doxiadis II, Levering WH, Roelen DL, Claas FH, Brand A. HLA-DRB1 associations in individuals with single and multiple clinically relevant red blood cell antibodies. *Transfusion*. 2014;54(8):1971-1980.
- Tatari-Calderone Z, Tamouza R, Le Boudier GP, et al. The association of CD81 polymorphisms with alloimmunization in sickle cell disease. *Clin Dev Immunol*. 2013;2013:937846.
- Oliveira VB, Dezan MR, Gomes FCA, et al. -318C/T polymorphism of the CTLA-4 gene is an independent risk factor for RBC alloimmunization among sickle cell disease patients. *Int J Immunogenet*. 2017;44(5):219-224.
- Meinderts SM, Sins JWR, Fijnvandraat K, et al. Nonclassical FCGR2C haplotype is associated with protection from red blood cell alloimmunization in sickle cell disease. *Blood*. 2017;130(19):2121-2130.
- Zhong H, Bao W, Friedman D, Yazdanbakhsh K. Hemin controls T cell polarization in sickle cell alloimmunization. *J Immunol*. 2014;193(1):102-110.
- Godfrey E, Liu Y, Shi P, et al. Altered heme-mediated modulation of dendritic cell function in sickle cell alloimmunization. *Haematologica*. 2016;101(9):1028-1038.
- Vingert B, Tamagne M, Habibi A, et al. Phenotypic differences of CD4(+) T cells in response to red blood cell immunization in transfused sickle cell disease patients. *Eur J Immunol*. 2015;45(6):1868-1879.
- Godfrey E, Zhong H, Pham P, Friedman D, Yazdanbakhsh K. TIGIT-positive circulating follicular helper T cells display robust B-cell help functions: potential role in sickle cell alloimmunization. *Haematologica*. 2015;100(11):1415-1425.
- Bao W, Zhong H, Li X, et al. Immune regulation in chronically transfused allo-antibody responder and nonresponder patients with sickle cell disease and β -thalassaemia major. *Am J Hematol*. 2011;86(12):1001-1006.
- Bao W, Zhong H, Manwani D, et al. Regulatory B-cell compartment in transfused alloimmunized and non-alloimmunized patients with sickle cell disease. *Am J Hematol*. 2013;88(9):736-740.
- Vingert B, Tamagne M, Desmarests M, et al. Partial dysfunction of Treg activation in sickle cell disease [published correction appears in *Am J Hematol*. 2015;90(1):84]. *Am J Hematol*. 2014;89(3):261-266.
- Vichinsky EP, Luban NL, Wright E, et al; Stroke Prevention Trial in Sickle Cell Anemia. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. *Transfusion*. 2001;41(9):1086-1092.
- 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(2):190-193.
- 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(8):1732-1739.
- Chou ST, Jackson T, Vege S, Smith-Whitley K, Friedman DF, Westhoff CM. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. *Blood*. 2013;122(6):1062-1071.
- Silvy M, Tournamille C, Babinet J, et al. Red blood cell immunization in sickle cell disease: evidence of a large responder group and a low rate of anti-Rh linked to partial Rh phenotype. *Haematologica*. 2014;99(7):e115-e117.
- Noizat-Pirenne F, Lee K, Pennec PY, et al. Rare RHCE phenotypes in black individuals of Afro-Caribbean origin: identification and transfusion safety. *Blood*. 2002;100(12):4223-4231.
- Yazdanbakhsh K, Ware RE, Noizat-Pirenne F. Red blood cell alloimmunization in sickle cell disease: pathophysiology, risk factors, and transfusion management. *Blood*. 2012;120(3):528-537.
- 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(2):381-388.
- Floch A, Gien D, Tournamille C, et al. High immunogenicity of red blood cell antigens restricted to the population of African descent in a cohort of sickle cell disease patients [published online ahead of print 29 April 2018]. *Transfusion*. doi:10.1111/trf.14633.
- Noizat-Pirenne F, Bachir D, Chadebech P, et al. Rituximab for prevention of delayed

- hemolytic transfusion reaction in sickle cell disease. *Haematologica*. 2007;92(12):e132-e135.
44. Larson PJ, Lukas MB, Friedman DF, Manno CS. Delayed hemolytic transfusion reaction due to anti-Go(a), an antibody against the low-prevalence Gonzales antigen. *Am J Hematol*. 1996;53(4):248-250.
 45. Noizat-Pirenne F, Habibi A, Mekontso-Dessap A, et al. The use of rituximab to prevent severe delayed haemolytic transfusion reaction in immunized patients with sickle cell disease. *Vox Sang*. 2015;108(3):262-267.
 46. 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(1):91-92.
 47. Nickel RS, Hendrickson JE, Fasano RM, et al. Impact of red blood cell alloimmunization on sickle cell disease mortality: a case series. *Transfusion*. 2016;56(1):107-114.
 48. Narbey D, Habibi A, Chadebech P, et al. Incidence and predictive score for delayed hemolytic transfusion reaction in adult patients with sickle cell disease. *Am J Hematol*. 2017;92(12):1340-1348.
 49. Tormey CA, Stack G. The persistence and evanescence of blood group alloantibodies in men. *Transfusion*. 2009;49(3):505-512.
 50. Frimat M, Tabarin F, Dimitrov JD, et al. Complement activation by heme as a secondary hit for atypical hemolytic uremic syndrome. *Blood*. 2013;122(2):282-292.
 51. Chadebech P, Habibi A, Nzouakou R, et al. Delayed hemolytic transfusion reaction in sickle cell disease patients: evidence of an emerging syndrome with suicidal red blood cell death. *Transfusion*. 2009;49(9):1785-1792.
 52. Sagiv E, Fasano RM, Luban NLC, et al. Glucose-6-phosphate-dehydrogenase deficient red blood cell units are associated with decreased posttransfusion red blood cell survival in children with sickle cell disease. *Am J Hematol*. 2018;93(5):630-634.
 53. Gardner K, Hoppe C, Mijovic A, Thein SL. How we treat delayed haemolytic transfusion reactions in patients with sickle cell disease. *Br J Haematol*. 2015;170(6):745-756.
 54. Mekontso Dessap A, Pirenne F, Razazi K, et al. A diagnostic nomogram for delayed hemolytic transfusion reaction in sickle cell disease. *Am J Hematol*. 2016;91(12):1181-1184.
 55. de Montalembert M, Dumont M-D, Heilbronner C, et al. Delayed hemolytic transfusion reaction in children with sickle cell disease. *Haematologica*. 2011;96(6):801-807.
 56. Win N, Sinha S, Lee E, Mills W. Treatment with intravenous immunoglobulin and steroids may correct severe anemia in hyperhemolytic transfusion reactions: case report and literature review. *Transfus Med Rev*. 2010;24(1):64-67.
 57. Dumas G, Habibi A, Onimus T, et al. Eculizumab salvage therapy for delayed hemolysis transfusion reaction in sickle cell disease patients. *Blood*. 2016;127(8):1062-1064.
 58. Hayes C, Shafi H, Mason H, Klapper E. Successful reduction of plasma free-hemoglobin using therapeutic plasma exchange: a case report. *Transfus Apheresis Sci*. 2016;54(2):253-255.
 59. Louie JE, Anderson CJ, Fomani KFM, et al. Case series supporting heme detoxification via therapeutic plasma exchange in acute multiorgan failure syndrome resistant to red blood cell exchange in sickle cell disease. *Transfusion*. 2018;58(2):470-479.
 60. Schaer DJ, Buehler PW, Alayash AI, et al. Hemolysis and free hemoglobin revisited: exploring hemoglobin and hemin scavengers as a novel class of therapeutic proteins. *Blood*. 2013;121(8):1276-1284.
 61. Immenschuh S, Vijayan V, Janciauskiene S, Gueler F. Heme as a target for therapeutic interventions. *Front Pharmacol*. 2017;8:146.
 62. Nance ST. Global definitions of rare donors. *ISBT Sci Ser*. 2013;8(1):23-27.
 63. Gluckman E, Cappelli B, Bernaudin F, et al; Eurocord, the Pediatric Working Party of the European Society for Blood and Marrow Transplantation, and the Center for International Blood and Marrow Transplant Research. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. *Blood*. 2017;129(11):1548-1556.
 64. Allen ES, Srivastava K, Hsieh MM, et al. Immunohaematological complications in patients with sickle cell disease after haemopoietic progenitor cell transplantation: a prospective, single-centre, observational study. *Lancet Haematol*. 2017;4(11):e553-e561.
 65. Volt F. Red blood cell alloimmunisation in patients with sickle cell disease. *Lancet Haematol*. 2017;4(11):e506-e507.
 66. Ribeil JA, Hacein-Bey-Abina S, Payen E, et al. Gene therapy in a patient with sickle cell disease. *N Engl J Med*. 2017;376(9):848-855.