Red cell transfusion and alloimmunization in sickle cell disease

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Red cell transfusion remains a critical component of care for acute and chronic complications of sickle cell disease. Randomized clinical trials demonstrated the benefits of transfusion therapy for prevention of primary and secondary strokes and postoperative acute chest syndrome. Transfusion for splenic sequestration, acute chest syndrome, and acute stroke are guided by expert consensus recommendations. Despite overall improvements in blood inventory safety, adverse effects of transfusion are prevalent among patients with sickle cell disease and include alloimmunization, acute and delayed hemolytic transfusion reactions, and iron overload. Judicious use of red cell transfusions, optimization of red cell antigen matching, and the use of erythrocytapheresis and iron chelation can minimize adverse effects. Early recognition and management of hemolytic transfusion reactions can avert poor clinical outcomes. In this review, we discuss transfusion methods, indications, and complications in sickle cell disease with an emphasis on alloimmunization.

Introduction

Transfusion remains a central intervention for sickle cell disease (SCD), with most patients receiving one or more transfusions by adulthood. Prospective, randomized clinical trials support transfusion for primary and secondary stroke prevention, but for many other indications, treatment is based on expert consensus. Guidelines on transfusion management for SCD are limited by availability of well-designed studies. Thus, many recommendations are based on low or moderate quality evidence or expert consensus, as well as the balance of benefits and harm for any given intervention. While transfusion therapy reduces SCD-associated morbidity and mortality, attention to prevention and management of alloimmunization, hemolytic transfusion reactions, and iron overload is critical.

Goals of transfusion

Red cell transfusion improves oxygen-carrying capacity and symptoms of anemia. For SCD, it may be used to increase a patient’s hematocrit and/or to reduce endogenous production of red cells containing hemoglobin S (HbS). Episodic transfusions are used for preoperative preparation or treatment of acute complications. Chronic transfusion therapy is utilized when the goal is to sustain a lower HbS level, such as for primary or secondary stroke prevention. A standard goal is to maintain HbS levels ≤30% or to raise the hemoglobin to 10-12 g/dL depending on the transfusion indication. Raising the hemoglobin to levels greater than 10-12 g/dL is generally avoided to limit the risk of hyperviscosity.

Transfusion method

Red cell transfusions can be provided by simple or exchange transfusion. In pediatric patients, simple transfusions are dosed by volume (i.e., 10-15 mL/kg), while in adults simple transfusions are provided in units (i.e., 1-2 units). Simple transfusion is convenient, requires one point of peripheral venous access, and utilizes fewer red cell units. Additionally, simple transfusion does not require special...
ized personnel or devices. Drawbacks of simple transfusion include risks of volume overload and hyperviscosity. Simple transfusion invariably leads to iron overload over time, necessitating treatment with iron chelation or alteration in transfusion modality.

Red cell exchange (RCE) procedures involve removal of the patient’s red cells and replacement with cells from the donor. RCE can be provided via automated (erythrocytapheresis) or manual methods. Manual RCE is performed using a series of repeated phlebotomies and transfusions, is time-consuming, and provides less consistent control of fluid balance during the procedure. Erythrocytapheresis requires apheresis machines and operators with technical expertise and may only be available at specialty centers. Since RCE typically replaces one or two times the patient’s total red cell volume, a higher volume of replacement cells is required. Despite increased exposure to donors with RCE compared to simple transfusion, several studies have shown no increase in alloimmunization rates associated with RCE. Limited studies indicate that RCE is cost-effective and may decrease hospitalization rates. Red cell replacement volume and hematocrit can be tightly controlled with RCE, allowing for significant reductions in HbS levels, while minimizing or preventing iron loading.

Erythrocytapheresis requires draw and return lines. Venous access must allow a steady flow of blood and withstand the high negative pressures of the draw line. Most adult patients have an adequate peripheral venous access to support RCE, but smaller pediatric patients often require central venous access. Indwelling catheters incur additional risks of infection and thromboembolic events.

RCE can be further modified to include isovolemic hemodilution, a process that includes initial removal of the patient’s red cells and replacement with normal saline or albumin followed by RCE. RCE with isovolemic hemodilution is not recommended in patients with recent or severe cerebrovascular or cardiopulmonary disease. Potential benefits of isovolemic hemodilution include improved efficiency of RCE, reduced number of red cell units per exchange, and decreased procedure frequency, however a recent meta-analysis found little evidence to support the use of RCE with isovolemic hemodilution over RCE without isovolemic hemodilution.

RCE is recommended over simple transfusion for acute ischemic stroke, severe acute chest syndrome, for patients with high baseline hematocrits requiring transfusion, and for chronically transfused patients with significant iron overload. Guidelines published by the American Society of Hematology (ASH) suggest using automated RCE in all patients with SCD receiving chronic transfusion therapy; however, individualized decisions for patients should consider availability of compatible red cell units and venous access.

**General transfusion considerations**

Prior to transfusion, an extended red cell antigen profile, including typing for C/c, E/e, K/k, Fy\(^a\)/Fy\(^b\), Jk\(^a\)/Jk\(^b\), M/N, and S/s, should be obtained for all patients with SCD. An antigen profile performed by genotyping is preferred, as it provides increased accuracy for C and Fy\(^b\) antigen expression in this population. Serological pheno-typing may be inaccurate if the patient has been transfused in the preceding 3 months. Extended red cell antigen profiles guide antigen matching and evaluation of positive antibody screens.

It is critical to obtain a patient’s antibody history from all hospitals that provided prior transfusions. The majority of antibodies are not detectable 6 to 12 months after initial identification. Knowledge of antibody history is necessary to avoid re-exposure to implicated antigens and reduce risk of hemolytic transfusion reactions.

Leukocyte reduction decreases the transmission of cytomegalovirus as well as the occurrence of HLA alloimmunization and febrile non-hemolytic transfusion reactions and is standard practice at most transfusion services treating patients with SCD. Irradiation prevents transfusion-associated graft-versus-host disease and is required for patients undergoing hematopoietic stem cell transplantation. Patients with SCD should receive transfusions negative for sickle cell trait. This aids accurate monitoring of post-transfusion HbS levels, a parameter utilized in chronic exchange programs and when assessing possible delayed hemolytic transfusion reactions (DHTR).

**Indications for transfusion in sickle cell disease**

Transfusions are a key component of managing SCD-associated complications (Table 1). Patients can experience acute exacerbations of anemia due to parvovirus-induced red cell aplasia, splenic and hepatic sequestration, and vaso-occlusive episodes. Transfusion therapy should be based on symptomatic anemia and hemodynamic compromise rather than hemoglobin value. Transfusion is also utilized to decrease the HbS level rapidly in patients experiencing stroke, acute chest syndrome (ACS), and multiorgan failure. The benefit of transfusion has not been well studied for pulmonary hypertension, priapism, and leg ulcers. Transfusion is not indicated for uncomplicated vaso-occlusive episodes.

**Neurological complications**

Cerebrovascular accidents are a significant source of morbidity and mortality in patients with SCD. Prior to implementation of routine screening, between 4-11% of patients experienced a stroke within the first two decades of life, and, without further therapy, two-thirds of patients developed recurrent stroke within 36 months. The landmark Stroke Prevention Trial in Sickle Cell Anemia (STOP trial) identified children at high risk of stroke using transcranial Doppler to detect elevated internal carotid or middle cerebral artery blood flow velocity. Among a randomized cohort of 150 patients, those receiving chronic transfusion therapy had a 92% lower risk of stroke than those in the standard-of-care arm. In the STOP II study, children whose transcranial Doppler findings had normalized after receiving transfusion therapy for 30 months were randomized to continue or stop chronic transfusion therapy. The study was terminated early after a significant proportion of the children who stopped receiving transfusions developed high-risk transcranial Doppler findings or overt stroke in contrast to none of the children in the continued-transfusion arm. Discontinuing transfusions in the STOP II trial
was also associated with higher occurrence of silent cerebral infarcts.23

More recently, the Transcranial Doppler with Transfusions Changing to Hydroxyurea (TWiTCH) trial explored transitioning patients with abnormal transcranial Doppler findings but no severe vasculopathy and at least 1 year of chronic transfusions to hydroxyurea versus continuation of chronic transfusions.26 Neither treatment group developed new stroke or evidence of new cerebral infarcts on magnetic resonance imaging, and hydroxyurea therapy at maximum tolerated dose was determined to be non-inferior to standard transfusions. Hydroxyurea can be considered as an alternative therapy for selected patients on chronic transfusion for primary stroke prevention.

The Stroke With Transfusions Changing to Hydroxyurea (SWiTCH) study was a multicenter randomized trial comparing transfusions plus chelation to hydroxyurea and phlebotomy in pediatric patients with a history of stroke and iron overload.27 The primary composite endpoint of the study included quantitative liver iron content and stroke recurrence rate. The study closed early after interim analysis indicated that liver iron content was not significantly different between groups. Importantly, although within the range of the study’s non-inferiority margin, there was an imbalance of seven strokes in the hydroxyurea arm compared to no strokes in the subjects receiving transfusions.

Silent cerebral infarcts are common in children with SCD and are associated with cognitive deficits and poor educational attainment.23 A history of silent cerebral infarcts predicts an increased risk of recurrent infarct, in the form of both other silent cerebral infarct and overt stroke.23,24 The Silent Infarct Transfusion trial showed that chronic transfusion therapy reduced the incidence of recurrent cerebral infarction in children with SCD.25 However, this study did not compare the efficacy of hydroxyurea to chronic transfusion, so implementation of chronic transfusion for patients with silent cerebral infarcts has not been robust given the availability of hydroxyurea and the burdens of chronic transfusion therapy.

Erythrocytapheresis is the preferred transfusion modality for acute stroke given its ability to decrease HbS levels rapidly while limiting effects on serum viscosity. In settings of both acute cerebral ischemia and stroke prevention, maintenance of HbS level ≤30% has been the standard of care.26

**Acute chest syndrome**

Acute chest syndrome (ACS) is one of the most common complications of SCD and is a leading cause of hospitalization and death.27 ACS is defined as a new pulmonary infiltrate on chest radiograph in the presence of respiratory symptoms, hypoxia, chest pain, or fever. Episodes can be triggered by infection, fat embolism, atelectasis, and infarction.27 The clinical course and spectrum of the disease are variable. While studies defining standardized criteria to assess ACS severity are lacking, patients with significant hypoxia or rapidly declining hemoglobin are considered to have severe disease.2

Simple transfusion provided early in the course of moderate ACS often prevents progression of the disease and the need for RCE.28-31 The ASH 2020 guidelines recognized the paucity of large-scale studies but suggest RCE over simple transfusion for patients with severe ACS, rapidly progressive ACS, or ACS in patients with high baseline hemoglobin.2

Recurrent episodes of ACS can lead to chronic lung disease including pulmonary hypertension and fibrosis. Hydroxyurea is the primary treatment for prevention of

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**Table 1.** Summary of indications for transfusion therapy in patients with sickle cell disease.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Transfusion method</th>
<th>Level of support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient aplastic crisis</td>
<td>Simple</td>
<td>Expert consensus</td>
</tr>
<tr>
<td>Acute multisystem organ failure</td>
<td>Simple or exchange*</td>
<td>Expert consensus</td>
</tr>
<tr>
<td>Acute hepatic sequestration</td>
<td>Simple or exchange</td>
<td>Expert consensus</td>
</tr>
<tr>
<td>Acute splenic sequestration</td>
<td>Simple via small volume aliquots of 3-5 mL/kg</td>
<td>Expert consensus</td>
</tr>
<tr>
<td>Acute splenic sequestration, recurrent</td>
<td>Simple as a bridge to splenectomy</td>
<td>Expert consensus</td>
</tr>
<tr>
<td>Acute ischemic stroke</td>
<td>Exchange &gt; simple</td>
<td>Observational studies; expert consensus</td>
</tr>
<tr>
<td>Primary stroke prevention</td>
<td>Simple or exchange</td>
<td>Randomized clinical trial</td>
</tr>
<tr>
<td>Secondary stroke prevention</td>
<td>Simple or exchange</td>
<td>Randomized clinical trial</td>
</tr>
<tr>
<td>Moderate acute chest</td>
<td>Simple or exchange</td>
<td>Expert consensus</td>
</tr>
<tr>
<td>Severe acute chest</td>
<td>Exchange</td>
<td>Expert consensus</td>
</tr>
<tr>
<td>Acute chest, recurrent</td>
<td>Simple or exchange</td>
<td>Hydroxyurea preferable</td>
</tr>
<tr>
<td>Preoperative with &gt; 1 hour with general anesthesia</td>
<td>Simple or exchange</td>
<td>Randomized clinical trial</td>
</tr>
<tr>
<td>Pregnancy with complications</td>
<td>Simple or exchange</td>
<td>Expert consensus</td>
</tr>
<tr>
<td>Pregnancy, uncomplicated</td>
<td>Simple or exchange</td>
<td>Under investigation</td>
</tr>
<tr>
<td>Prior to hematopoietic stem cell transplant</td>
<td>Simple or exchange</td>
<td>Under investigation</td>
</tr>
<tr>
<td>Uncomplicated vaso-occlusive episode</td>
<td>---</td>
<td>Transfusion not recommended</td>
</tr>
<tr>
<td>Priapism</td>
<td>---</td>
<td>Transfusion not recommended</td>
</tr>
<tr>
<td>Leg ulcers</td>
<td>---</td>
<td>Transfusion not recommended</td>
</tr>
<tr>
<td>Avascular necrosis</td>
<td>---</td>
<td>Transfusion not recommended</td>
</tr>
</tbody>
</table>

*The British Committee for Standards in Haematology recommend exchange transfusion for acute multisystem organ failure. Red cell exchange may be preferred for severe, acute multisystem organ failure.
recurrent ACS. Analysis of patients in the STOP trial showed a significantly decreased incidence of ACS in the chronic transfusion arm, and small studies suggest it may reduce frequency of ACS recurrence but not severity.32,33 Chronic transfusion therapy can be considered in patients with recurrent ACS when hydroxyurea is not well tolerated or when hydroxyurea is insufficient to prevent severe, recurrent ACS.

Preoperative transfusion support

Operations, including cholecystectomy, splenectomy, and hip surgery, are common in patients with SCD and carry a significant risk of morbidity and mortality.34 The observational Cooperative Study of Sickle Cell Disease demonstrated that patients with SCD undergoing surgery had high rates of pain and ACS, as well as complications such as fever, bleeding, and death.34,35 The Transfusion Alternatives Preoperatively in Sickle Cell Disease (TAPS) study randomized patients with hemoglobin SS and Sβ-thalassemia SCD requiring low- or medium-risk operations to transfusion or no transfusion preoperatively.36 The rate of postoperative ACS was markedly reduced among patients in the transfusion arm. The majority (85%) of study patients underwent medium-risk operations and, therefore, the relevance of these findings to low-risk surgeries is uncertain. The effect of preoperative transfusion on postoperative pain crises was less clear. Multiple studies have found no significant reduction in postoperative pain in preoperatively transfused patients.2,36,37

Preoperative transfusion support for transplantation and curative therapies

There are limited studies examining transfusion considerations in patients with SCD undergoing allogeneic or autologous hematopoietic stem cell transplantation. Blood transfusion prior to transplantation may reduce SCD-associated bone marrow changes and inflammation, possibly improving transplant outcomes. Reducing HbS levels to ≤30% prior to transplantation may also minimize SCD-related complications in the peri-transplant period. Recent studies and ongoing clinical trials utilize

![Figure 1. Factors that contribute to alloimmunization in sickle cell disease.](image-url)
transfusion parameters of hemoglobin <10-11 g/dL and HbS percentage <30% prior to autologous stem cell mobilization and transplantation. The optimal transfusion modality and timing peri-transplant are important areas of further study.

Alloimmunization in sickle cell disease

Alloantigen specificity and donor/recipient antigen discrepancies

Alloimmunization, or formation of antibodies to non-self antigens, is a major adverse effect of transfusion. Alloimmunization increases the risk of hemolytic transfusion reactions and leads to delays in identification of compatible red cell units. While multilcenter, registry-based studies have identified a prevalence of red cell alloimmunization of 2-5% in the general population, the prevalence in patients with SCD ranges from 5-75%. 40-42 The pathophysiology of alloimmunization in SCD is complex and is associated with level of antigen matching, Rh blood group system diversity, and immune factors (Figure 1).

Antibodies to Rh system antigens C and E and to the Kell system antigen K historically accounted for up to two-thirds of alloantibodies in SCD. 43-44 Rh and Kell system antigens are among the most immunogenic. Thus, a primary driver behind alloimmunization is recipient-donor mismatch of Rh and K antigens. The majority of Blacks lack C and E antigens (75% and 78%, respectively), and only 2% express the K antigen. 45 In the predominately white blood donor populations in the USA and Europe, the frequencies of C, E, and K are higher, leading to recipient-donor mismatch. 46,47 Other clinically relevant antigens, including Jk^k in the Kidd system, Fy^a in the Duffy system, and S in the MNS system are also more frequent in all patients with SCD at the earliest opportunity. 2

Red cell antigen matching

The British Society for Haematology guidelines, the ASH 2020 guidelines, and the National Institutes of Health Expert Panel recommend prophylactic matching for Rh (C, E or C/c, E/e), and K in addition to ABO and D in patients with SCD. 4,45 Transfusion of red cells matched for Rh and K decreases the rate of alloimmunization from 1.7-3.9 antibodies per 100 units transfused to 0.26-0.50 antibodies per 100 units transfused. 48 Additional antigen matching extended for Fya/Fyb, Jka/Jkb, M/N, S/s, Lea/Leb, and P antigens further reduces the rate of alloantibody formation to 0.1-0.3 per 100 units transfused, but finding sufficient compatible units becomes significantly more challenging. 49,50

An extended red cell antigen profile should be obtained in all patients with SCD at the earliest opportunity. 2 Extended antigen identification has traditionally been performed by manual serological phenotyping methods. As most blood group antigens are due to single nucleotide polymorphisms, high throughput genotyping systems can identify Rh, Kell, Kidd, Duffy, MNS, Lutheran, Diego, Dombrock, and Colton blood group system antigens. 51 DNA-based red cell typing is more accurate than serological phenotyping and provides additional information, such as whether a patient has a GATA mutation in the ACKR1 gene and is, therefore, not at risk of forming anti-Fy^a antibodies. 52 Genotyping methods also provide increased accuracy for C antigen expression in this population, predict antigen expression when no antisera is available, and should be used over serological phenotyping if the patient has been transfused in the preceding 3 months. Several studies have utilized molecular genotyping to support extended antigen matching between blood donors and patients with SCD. 4,53

RH diversity and the role of RH genotyping

Despite serological matching for D and C, E or C/c, E/e antigens, Rh alloimmunization persists due to RH genetic diversity in individuals of African descent. 53,54 The RH and RHCE genes are located on chromosome 1, arose through gene duplication, and encode D and C, c, E, e antigens, respectively. 55 The two loci are highly homologous, leading to many gene recombination events resulting in variant RH and RHCE alleles that encode altered antigens. 56,57 Rh variant antigens are difficult to distinguish serologically and require RH genotyping for identification. While RH variants can result in weak (decreased antigen density) or partial (missing epitopes) antigen expression, Blacks typically carry alleles in the latter category and are at risk of alloantibody formation when exposed to the epitopes they lack via transfusion, pregnancy, or transplantation. High suspicion must be maintained when apparent autoantibodies with Rh specificity or unexplained Rh antibodies are detected in patients with SCD, and further investigation with RH genotyping should be pursued.

Most patients with SCD have one or more RH allele variants. 49,53-55 Two common variants in Blacks, RH*D^AUA and RHCE^ce48C, have not been shown to encode Rh proteins lacking epitopes and are considered “altered” antigens. 54 RH^D 4.0, *DIVa, *DAU5, and *DIIla are frequently detected variants in patients with SCD and result in partial D antigen expression. 51,62

The hybrid RH*DIIla-CE(4-7)-D allele results from RHCE exons 4 through 7 replacing the corresponding exons of RHD. This allele encodes a partial C antigen and no D antigen. Individuals with RH*DIIla-CE(4-7)-D who lack conventional RHCE^Ce or *CE alleles serologically type as C positive but are at risk of developing alloanti-C if exposed to conventional C antigen. 56 Variant RhCE antigens resulting in partial c and e antigens are particularly common in Blacks. Individuals with homozygous ce variants often make allo-anti-e antibodies and may also lack the high frequency hr^ and hr^ negative, can facilitate proper antibody evaluation and distinguish these antibodies from anti-e. E-e+ patients with partial e antigens who form allo-anti-e are at risk of anti-E if transfused with E-e- red cells. While each clinical scenario requires individual decision-making, if there was no associated DHTR
with the anti-e, we have cautiously transfused patients with e+ blood without evidence of a DHTR or anti-e re-appearance. In the future, RH genotyping matched red cells would be the ideal choice.

The role of RH genotyping in blood donors and patients with SCD and genotype matching to prevent alloimmunization is currently under investigation. While systematic RH genotyping of patients with SCD may aid in blood product selection and reduce the risk of alloimmunization in patients with variant RH alleles, a large pool of genotyped Black donors would be needed as well. Universal RH genotyping is currently cost-prohibitive in most settings, but one study has shown that prophylactic RH matching based on genotype for patients with SCD is achievable but would require recruitment of double the number of Black blood donors as compared to those for serological matching.54

Inflammation and immune system regulation in alloimmunization

A subset of patients with SCD do not form alloantibodies despite repeated transfusions. Genetic modifiers and differences in immune regulation likely contribute to an individual’s risk. HLA molecules present foreign red cell antigens to T cells. Activated CD4+ T cells stimulate B-cell responses and differentiation into plasma cells. The class II HLA alleles HLA-DR2, HLA-DR3, and HLA-DR5 are associated with lower risk of red cell alloimmunization, while HLA-DR7, HLA-DRB1*04, and HLA-DRB1*15 may be associated with increased risk.65,66 Efforts to identify genetic markers for alloimmunization in patients with SCD have demonstrated moderate associations but no strong predictors.65,66

Patients with high baseline levels of inflammatory cytokines, such as those with autoimmune disease, have higher rates of alloimmunization.65 SCD is a chronic inflammatory state in which hemolysis results in elevated levels of circulating hemoglobin and free heme, activating neutrophils and macrophages and causing secretion of pro-inflammatory cytokines. Accordingly, patients with SCD have higher levels of pro-inflammatory cytokines, including interleukin-1, interleukin-6, and interferon-γ, as compared to the levels in healthy controls.69 Fasano and colleagues demonstrated that patients with SCD who received transfusions during inflammatory events such as ACS and vaso-occlusive episodes had an increased rate of alloimmunization.70

Chronic inflammation can lead to immune system dysregulation. Several studies have shown that regulatory T cells, which control T-cell responses, display higher levels of inhibitory markers such as CTLA-4 and are dysfunctional in patients with SCD.71 Furthermore, regulatory B cells from alloimmunized patients with SCD have a decreased ability to suppress monocyte activation.72 Pal et al.72 demonstrated that hemolysis and cell-free heme typically suppress B cells and plasma cell differentiation, but alloimmunized patients with SCD have altered B-cell inhibition. Further mechanistic studies are required to elucidate the complex immunological pathways contributing to alloimmunization in SCD and to determine whether targeted reversal of immune dysregulation can reduce antibody formation.

Clinical impact of alloimmunization

Red cell alloimmunization in patients with SCD significantly increases the risk of hemolytic transfusion reactions, including hyperhemolytic reactions. Identifying compatible blood for patients with multiple alloantibodies or antibodies to high prevalence antigens can be challenging or even impossible, potentially leading to transfusion delays and poor outcomes.

Additional complications of packed red blood cell transfusion

Delayed hemolytic transfusion reactions and hyperhemolysis

DHTR is a feared adverse outcome of transfusion in SCD.34 DHTR classically occurs after re-exposure to a red cell antigen that the patient had previously been immunized against. As many as 80% of alloantibodies in patients with SCD become undetectable.69 In patients with SCD, 30-40% of DHTR are associated with no identifiable antibodies, and in one-third of cases, autoantibodies or antibodies of unclear specificity are the only detectable finding.67,69 The mechanisms of red cell destruction in antibody-negative DHTR have not been elucidated, however hypotheses include hyperactivated macrophages and red cells with increased membrane exposure of phosphatidylserine.70,71 Alternatively, the antibody may simply be difficult to detect. The most severe complication is hyperhemolysis, in which hemolysis of bystander autologous cells occurs, leading to a hemoglobin level lower than pre-transfusion levels and often life-threatening anemia.

The reported incidence of DHTR in SCD is 4.8-7.7%.34,76 These rates may be underestimates, as many DHTR are misdiagnosed as vaso-occlusive episodes or go undetected. In one of the largest cohorts to date, the most common clinical manifestations of DHTR were hemoglobinuria, pain, and fever.71 Only 44% of patients had overt signs of anemia. Signs and symptoms of DHTR vary among individuals. The recent ASH guidelines define DHTR as a significant drop in hemoglobin level within 21 days after transfusion in the presence of hemoglobinuria, newly detected alloantibodies, accelerated increase in HbS, significant drop in reticulocyte percentage, or increase in lactate dehydrogenase level above baseline.71 Rapid decline in HbA concentration relative to an early post-transfusion measurement is highly predictive of DHTR. Risk factors include a history of alloimmunization, prior DHTR, and transfusion for acute complications.76,80,82

High suspicion must be maintained when patients with SCD present with pain, fever, or worsening anemia in the days to weeks after transfusion. Habibi et al. reported that 92% of patients with DHTR were not immediately diagnosed.82 Review of the transfusion history is required, and an antibody screen, direct antiglobulin test (DAT) with elution, and hemoglobin electrophoresis should be performed if the patient has been recently transfused. Between 25-60% of DHTR are associated with newly detected red cell antibodies, and approximately 80% of DHTR are DAT positive.55,57,58 Time to DAT positivity is variable, and it is recommended that the DAT and antibody screen are repeated 1-2 weeks after presentation in cases of DHTR that are initially antibody-negative.

Additional transfusions may exacerbate hemolysis, particularly when the antibody is not identified. Upon recognition of a DHTR, further transfusion should be avoided if possible. If transfusion is necessary and no antibody specificity has been identified, extended antigen matching for
C/c, E/e, K, Jk'/Jk, Fy/Fy, and S/s is recommended. Many patients improve with hydration, oxygen support, and pain management alone, but others develop severe complications such as ACS and multiorgan failure. Erythropoietin with or without intravenous iron is an additional supportive measure. High-dose steroids and intravenous immunoglobulins are suggested first-line therapy for patients with severe DHTR or ongoing hemolysis, although caution must be maintained, as high-dose steroids have been associated with rebound symptoms and worsening vaso-occlusive episodes. Prophylactic treatment with steroids and intravenous immunoglobulins prior to transfusion should be considered for patients with a history of multiple or life-threatening DHTR or for those for whom compatible blood is not available. Rituximab, an anti-CD20 monoclonal antibody, can be used to reduce the risk of further alloimmunization when future transfusion is likely and may be employed as a prophylactic therapy prior to transfusion for patients with a history of multiple or severe DHTR.

There is growing evidence that the complement pathway plays a pivotal role in the pathogenesis of DHTR. Alloantibody-antigen complexes activate the classical complement pathway. Free heme and hemoglobin trigger the alternative complement pathway, leading to endothelial damage and organ injury. Eculizumab, a monoclonal anti-C5 antibody targeting terminal complement activation, has been used as salvage therapy in cases of severe DHTR and hyperhemolysis. Case reports have also described treatment of hyperhemolysis with tocilizumab, a monoclonal antibody against the interleukin-6 receptor. These cases showed marked improvement after targeted anti-interleukin-6 receptor therapy, suggesting that blockade of macrophage activation may be an effective treatment strategy. Table 2 summarizes published reports describing use of eculizumab and tocilizumab in patients with SCD.

DHTR are associated with high mortality, underscoring the importance of early recognition and treatment, but prevention is key. Red cell exposure should be minimized by transfusing only for evidence-based indications. All patients should be prophylactically matched for Rh (C, E or C/c, E/e) and K antigens. Patients at high risk of DHTR should be identified and transfusions avoided or delivered with immunomodulatory agents as possible. Incomplete transfusion and alloantibody histories contribute to the incidence of DHTR. Most countries lack national transfusion databases, so patients should be encouraged to make new providers aware of their transfusion history and limit transfusions to one institution, if possible.

### Iron overload

Each milliliter of transfused red cells contains 0.8-1 mg of iron. Transfusion of 3-5 units of packed red cells delivers 1 g of iron, a significant burden considering the total body iron of an average adult is 4-5 g. The human body has no mechanisms for excreting excess iron. While small amounts of iron are lost through the gastrointestinal tract and skin, iron homeostasis is primarily regulated by hepcidin, a protein synthesized by the liver in response to iron overload and inflammation. Hepcidin inhibits dietary iron absorption and blocks iron recycling through the reticuloendothelial system. Transfusional iron is delivered outside of these normal regulatory mechanisms, and there are no means of eliminating large amounts of iron from transfusion.

In SCD, iron accumulation is most prominent in the liver. Compared to thalassemia patients with equivalent transfusion volumes, patients with SCD are less vulnerable to iron overload-induced endocrinopathies and heart failure; however, iron cardiomyopathy is detectable in 2.5% of SCD patients receiving chronic transfusion therapy. Iron toxicity is estimated to contribute to 7-11% of deaths in patients with SCD.

### Table 2. Reports of studies investigating eculizumab and tocilizumab for the treatment of delayed hemolytic transfusion reactions and hyperhemolysis in patients with sickle cell disease.

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug investigated</th>
<th>Dose</th>
<th>Number of patients</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boonyasampant et al. 2015</td>
<td>Eculizumab</td>
<td>1200 mg weekly x 4 weeks followed by every 2 weeks for 2 more doses</td>
<td>1</td>
<td>None reported</td>
</tr>
<tr>
<td>Dumas et al. 2016</td>
<td>Eculizumab</td>
<td>900 mg x 2 dosed 1 week apart</td>
<td>3</td>
<td>1 death secondary to severe pulmonary infection</td>
</tr>
<tr>
<td>Chonat et al. 2018</td>
<td>Eculizumab</td>
<td>600 mg x 2</td>
<td>1</td>
<td>None reported</td>
</tr>
<tr>
<td>Vlachaki et al. 2018</td>
<td>Eculizumab</td>
<td>900 mg x 1</td>
<td>1</td>
<td>None reported</td>
</tr>
<tr>
<td>Unnikrishnan et al. 2019</td>
<td>Eculizumab</td>
<td>900 mg x 1</td>
<td>1</td>
<td>None reported</td>
</tr>
<tr>
<td>Chonat et al. 2020</td>
<td>Eculizumab</td>
<td>600 mg weekly x 4 weeks</td>
<td>1</td>
<td>None reported</td>
</tr>
<tr>
<td>Foch et al. 2020</td>
<td>Eculizumab</td>
<td>1-3 doses</td>
<td>18</td>
<td>3 patient deaths (2 from complications of encapsulated bacterial infection)</td>
</tr>
<tr>
<td>Mpinganzima et al. 2020</td>
<td>Eculizumab</td>
<td>900 mg x 2 dosed 6 days apart</td>
<td>1</td>
<td>None reported</td>
</tr>
<tr>
<td>Sivapalaratnam et al. 2019</td>
<td>Tocilizumab</td>
<td>8 mg/kg daily x 2 days</td>
<td>1</td>
<td>None reported</td>
</tr>
<tr>
<td>Lee et al. 2020</td>
<td>Tocilizumab</td>
<td>8 mg/kg daily x 4 days</td>
<td>1</td>
<td>Seizure (in the setting of methemoglobinemia secondary to hemoglobin-based oxygen carrier)</td>
</tr>
<tr>
<td>Hair et al. 2021</td>
<td>Eculizumab and Tocilizumab</td>
<td>900 mg x 3, 8 mg/kg x 1</td>
<td>1</td>
<td>None reported</td>
</tr>
</tbody>
</table>
Global challenges in transfusion support for sickle cell disease

The worldwide incidence of SCD is highest in sub-Saharan Africa, accounting for approximately 75% of the global burden of SCD.\textsuperscript{106,107} Although red cell transfusion significantly reduces morbidity and mortality associated with SCD, transfusion support in sub-Saharan Africa is limited by the availability and safety of blood products. While 15% of the global population resides in sub-Saharan Africa, only 4% of blood donations occur in this region.\textsuperscript{108} Blood donations in Africa have increased over the past decade, but widespread blood shortages remain.\textsuperscript{109} The high cost of blood products in these regions poses a further challenge.\textsuperscript{109} Red cell and whole blood transfusions in patients with SCD in sub-Saharan Africa are often restricted to patients with acute complications. Transcranial Doppler screening is not widely available, and chronic transfusion therapy is often unattainable.

Several studies have supported higher rates of transfusion reactions in regions of Africa compared to those in higher-resource regions, attributable to factors including limited implementation of leukoreduction, challenges in maintaining temperature control during storage of blood products, and need for effective quality and transfusion education systems.\textsuperscript{109,110} While most countries in Africa routinely screen blood products for human immunodeficiency virus, hepatitis B, and hepatitis C, the residual risk of transfusion-transmitted viral infection is relatively high, particularly in countries with higher percentages of paid or family/replacement blood donors.\textsuperscript{108,111} Transfusion-transmitted malaria and emerging infectious diseases pose additional burdens.

Pre-transfusion testing in sub-Saharan Africa typically comprises ABO and D typing and saline crossmatching. Antiglobulin reagents are in limited supply, and antibody screening is not consistently performed in most settings.\textsuperscript{109,112} Although pre-transfusion antigen typing and prophylactic antigen matching are not routinely available in low-income countries, rates of alloimmunization among patients with SCD in sub-Saharan Africa may be equivalent to or lower than those in higher or middle income countries.\textsuperscript{112} It is hypothesized that reduced transfusion rates and greater antigenic similarity between donor and recipient populations contribute to these findings, although further studies are required.

Efforts to improve transfusion support for patients with SCD living in lower-resource countries are paramount and, along with other measures, such as increasing availability of hydroxyurea, would undoubtedly improve care and quality of life for the majority of patients with SCD.

### Table 3. Characteristics of iron chelators.

<table>
<thead>
<tr>
<th>Iron Chelator</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deferasirox (Exjade, Jadenu)</strong></td>
<td><strong>Deferoxamine (Desferal)</strong></td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td>Oral</td>
</tr>
<tr>
<td></td>
<td><strong>[Jadenu: coated tablet or sprinkles]</strong></td>
</tr>
<tr>
<td></td>
<td><strong>[Exjade: dispersible tablet]</strong></td>
</tr>
<tr>
<td><strong>Route of excretion</strong></td>
<td>Fecal</td>
</tr>
<tr>
<td><strong>Toxicities</strong></td>
<td>Gastrointestinal upset, proteinuria, renal dysfunction, raised transaminases, gastrointestinal bleeding (rare)</td>
</tr>
</tbody>
</table>

Regular assessment of iron overload is recommended for patients with SCD receiving chronic transfusion therapy.\textsuperscript{2} While serum ferritin levels are widely available, relatively inexpensive, and can be easily serially monitored, ferritin is an acute phase reactant and its levels do not always correlate with total body iron stores. Magnetic resonance imaging is currently the recommended technique for quantifying liver iron.\textsuperscript{101,102} Both R2 and R2* magnetic resonance imaging data show strong correlation with iron levels on liver biopsy but are not interchangeable, so the same method should be used to monitor a patient longitudinally.\textsuperscript{103} Regular assessment of liver iron concentration by liver magnetic resonance imaging every 1-2 years is recommended for chronically transfused patients with SCD or those with sustained serum ferritin levels \(>1000\) ng/mL.\textsuperscript{2} Given the rarity of cardiac iron overload in SCD, routine screening for cardiac iron levels by T2* magnetic resonance imaging is recommended only for patients with evidence of cardiac dysfunction or a severe iron overload (liver iron content >15-20 mg/g).\textsuperscript{2}

Iron chelation is recommended for patients on chronic transfusion therapy who have sustained serum ferritin levels \(>1000\) ng/mL or liver iron content \(>3-7\) mg/g liver dry weight (normal range 0.8-1.5 mg/g liver dry weight).\textsuperscript{102} There are currently three iron chelators licensed and approved for use in Europe and the USA, all of which have shown to be effective in mitigating iron overload in patients with hemoglobinopathies (Table 3).\textsuperscript{103-104} Successful chelation therapy is dependent upon the patients’ adherence and the tolerability and toxicities of the drugs used.

Chronic transfusion modality and transfusion parameters can be modulated to reduce iron loading. In erythropheresis, post-procedure hematocrit can be targeted to a value equal to or lower than the pre-procedure hematocrit to maintain a neutral or net negative iron balance. For patients with transfusion-related anemia, transfusion to RCE due to iron overload, targeting a slightly lower hematocrit at the end of the procedure than the pre-transfusion hematocrit will reduce total body iron stores over time. Chelation therapy can be combined with RCE for greater reduction in liver iron content for iron-overloaded patients requiring chronic transfusions.\textsuperscript{105}
Future directions

Transfusion therapy is a cornerstone of treatment in SCD. Clinical trials have proven that transfusions are effective means of preventing stroke, decreasing postoperative ACS, and reducing morbidity and mortality in SCD. Given the risks of iron overload, alloimmunization, and DHTTR, transfusion should be used judiciously for evidence-based indications or those defined by expert consensus. Despite increased understanding of the pathophysiology of alloimmunization in SCD and improved execution of Rh and K antigen matching, high rates of alloimmunization persist. Future work is necessary to determine whether extended antigen matching or prophylactic RH genotype matching can reduce alloimmunization in a cost-effective manner. Recruitment of diverse donors and broad implementation of donor genotyping will increase compatible donors for patients with SCD. The need to improve safety and ensure access to reliable transfusion therapy for patients with SCD worldwide remains.

Disclosures
No conflicts of interest to disclose.

Contributions
GEL and STC wrote the manuscript.

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