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Managing alloimmunization, delayed hemolytic transfusion reactions, and hyper-hemolysis in sickle cell disease

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Thesis

**MANAGING ALLOIMMUNIZATION, DELAYED HEMOLYTIC
TRANSFUSION REACTIONS, AND HYPER-HEMOLYSIS IN SICKLE CELL
DISEASE**

by

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ABSTRACT

Sickle cell disease (SCD) is an inherited blood disorder in which polymerization of a mutated form of hemoglobin results in a sickled shape of red blood cells, leading to anemia due to premature destruction of RBCs. Other morbidities include end-organ damage, pain attacks known as “sickle cell crisis,” bacterial infections, and stroke. Transfusion therapy remains a necessary treatment of sickle cell disease. Transfusions given to lower the levels of sickle RBCs and viscosity have shown to decrease the risk of stroke by 90% during clinical trials.

However, chronically transfused patients can become alloimmunized and develop delayed hemolytic transfusion reactions (DHTR), a life-threatening condition. The presentation of DHTR can vary, and its severity is unpredictable. In the most severe cases, patients destroy their own RBCs along with the transfused RBCs, a condition known as hyper-hemolysis. Additional transfusions can aggravate these symptoms and lead to death. Clinicians’ awareness of DHTR events is poor because its clinical presentations mimic those of vaso-occlusive crisis. Furthermore, immunohematology at times detect no newly formed antibodies. Mortality due to DHTR have been reported to be as high as 11.5%.

Currently, 5.4 million people suffer from sickle cell disease. An overwhelming

80% of SCD occurs in Sub-Saharan Africa, while also occurring frequently in people of African origin living in other parts of the world. Annually, over 100,000 patients are affected by SCD, utilizing over one billion dollars per year in healthcare costs. Lastly, current projections estimate that by 2050 the number of newborns with sickle cell disease will exceed 400,000.

While there are no drugs that specifically target DHTR, immunosuppressants such as intravenous immunoglobulins, steroids, eculizumab, and tocilizumab have shown to improve outcomes in DHTR. However, these treatments may prove to be insufficient. Moreover, numerous adverse health effects are associated with using immunosuppressants, including exacerbation of vaso-occlusive pain and hemolytic anemia already present in SCD patients. Currently, alloimmunization can be prevented by extended-matching of blood antigen, increasing blood donations from African Americans and other minority groups, and the use of rituximab. While hematopoietic stem cell transplantation remains a viable alternative to transfusing therapy, it remains highly under-utilized.

This literature review aims to evaluate the current prevention and treatment methods used to manage DHTR to expose gaps in knowledge and identify ways to improve clinical outcomes in SCD patients. Results suggest:

(1) While it is not cost-effective to implement extended matching in all SCD patients, there is potential in strategies to increase blood donation in minority groups. Genotyping of blood antigens may also be considered.

(2) Treatment options are sorely lacking for DHTR. Case studies document

positive outcomes using immunosuppressants, but there are few clinical trials and evidence-based studies to confirm their efficacy in larger cohorts.

(3) Effective treatment of DHTR relies on prospective studies that further elucidate the pathophysiology of alloimmunization and DHTR.

(4) The lack of effective treatments for DHTR can be attributed to structural violence. Advocacy and awareness are instrumental in improving care for DHTR and SCD.

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LIST OF ABBREVIATIONS

ACS.....	acute chest syndrome
ARCET	emergency automated red cell exchange transfusion
DAT	antiglobulin test
EPO	erythropoietin
ET-1	endothelin-1
GVHD.....	graft versus host disease
Hb.....	hemoglobin
HbA.....	hemoglobin A
HbS	hemoglobin S
HLA	human leukocyte antigen
IL-6	interleukin-6
IVIg.....	intravenous immunoglobulins
HSCT	hematopoietic stem cell transplantation
LDH	lactate dehydrogenase
MOF.....	multiple organ failure
MP.....	methylprednisolone
NHIS	National Health Interview Survey
PGID	placenta growth factor
PNH.....	paroxysmal nocturnal hemoglobinuria
RBC.....	red blood cell

ROS.....reactive oxygen species
SES..... socioeconomic status
SCD..... sickle cell disease
SCT..... sickle cell trait
VOC..... vaso-occlusive crisis
WBC..... white blood cell

INTRODUCTION

Sickle cell disease (SCD) is an inherited blood disorder in which polymerization of a mutated form of hemoglobin results in a sickled shape of red blood cells (RBC), leading to anemia due to the premature destruction of RBCs. A single amino acid substitution (Glutamine to Valine) at the sixth position of the beta chain of hemoglobin S (HbS) results in HbS instability and polymerization, causing RBCs to form a rigid, sickle shape (Eaton & Bunn, 2017). The loss of RBC elasticity leads to adhesion-mediated vaso-occlusion, where deformed RBCs adhere to each other and block flow in small blood vessels. This results in devastating health complications such as end-organ damage, pain attacks known as “sickle cell crisis,” bacterial infections, and stroke. A person is known to have the sickle cell trait (SCT) when only one copy of the sickle cell gene is present (Telen et al., 2019). Individuals with SCT are able to lead normal lives without any SCD complications. Approximately 10% of American Americans (AA) and more than 100 million worldwide are estimated to carry the SCT (Yazdanbakhs et al., 2012)

Currently, 5.4 million people suffer from sickle cell disease, while 43 million have the sickle cell trait. An overwhelming 80% of sickle cell disease occurs in Sub-Saharan Africa, while also occurring frequently in people of African origin living in other parts of the world (Rees et al., 2010). Patients living in developed countries can expect to live beyond their 50s with adequate medical treatment. However, in low-income countries such as Africa, 90% of patients die before their fifth birthday (Gravitz & Pincock, 2014). In the United States, SCD prevalence is 1 in every 500 AA births, 1 in

every 36,000 Hispanic-American births, and 1 in every 100,000 Caucasian births (Hassell, 2010). Annually, over 100,000 patients are affected by SCD (Bunn, 1997), utilizing over one billion dollars per year in healthcare costs (Kauf et al., 2009). Lastly, current projections estimate that by 2050 the number of newborns with sickle cell disease will exceed 400,000 (Aslam et al., 2018).

Approaches to management of SCD can be separated into five categories: supportive, preventative, symptomatic, abortive, and curative therapy. Supportive management typically includes the essential components for good health, such as a balanced diet, sleep, hydration, and folic acid. Preventative management includes avoidance of stressful situations, vaccination, hemoglobin F induction with hydroxyurea, and transfusion. Symptomatic management aims to alleviate the symptoms as it occurs via transfusion, pain medication, and antibiotics (Ballas et al., 2012). Lastly, stem cell transplantation and gene therapy hold promising potential in reversing the pathophysiological process of SCD (Telen et al., 2019).

Blood transfusion is a life-saving treatment as it provides normal RBCs and increases oxygen distribution to tissues to decrease morbidity and mortality for SCD patients. Studies have demonstrated transfusions dramatically reduce the risk of stroke (Adams et al., 1998) and cerebral infarction (DeBaun et al., 2014) in children suffering from SCD. In clinical trials with SCD patients, transfusions to lower the percentage of sickle RBCs and blood viscosity decreased the risk of stroke by 90%.

60% to 90% of SCD patients will receive transfusions in their lifetime (Walter et al., 2009). However, chronic transfusions can lead to RBC alloimmunization in

approximately 20% to 50% of SCD patients (Yazdanbakhsh et al., 2012).

Alloimmunization occurs when patients develop antibodies against transfused RBCs.

These antibodies are usually directed against antigens expressed on RBCs of white persons, as they consist the majority of blood donors in Western countries (Vichinsky, 2001). Finding compatible units lacking these antigens can be time laborious and cause transfusion delays.

Delayed hemolytic transfusion reaction (DHTR) is the most life-threatening consequence of RBC alloimmunization. Among SCD patients receiving blood transfusions, between 4% to 11% have reported DHTR and its associated health effects. Its clinical presentations include acute hemolysis (hemoglobinuria, jaundice, and pallor), vaso-occlusive crisis (pain, fever, acute chest syndrome), anemia, and reticulocytopenia. The symptoms of DHTR present in a wide spectrum, and it is extremely difficult to predict its severity (Montalembert et al., 2011).

In the most severe case of DHTR, hyper-hemolysis can occur, a poorly understood condition where the transfused and patient's RBCs are destroyed (Yazdanbakhsh et al., 2012). Hemoglobin (Hb) level becomes lower than pre-transfusion levels, exacerbating anemia and SCD symptoms. Hyper-hemolysis can induce multiple organ failure (MOF) and death. Antibody screening detects alloantibodies (against blood groups) or autoantibodies (against self or unknown antigen). Interestingly, there are cases where antibodies are not detected. Furthermore, the frequency and mortality rates of DHTR may be underreported because DHTRs are commonly misdiagnosed as vaso-occlusive crises (VOC).

SPECIFIC AIMS

The uncertainty surrounding DHTR's exact mechanism of action complicates finding the best treatment approach. Currently, there are no guidelines to diagnose and treat DHTR with hyper-hemolysis. The standard treatment consists of corticosteroids, intravenous immunoglobins (IVIg), and avoidance of additional transfusions (Lee et al., 2019). Prevention of alloimmunization is especially challenging as there are cases of DHTR where antibodies are not detected. Given these complications and challenges in treating DHTR with hyper-hemolysis in SCD patients, this literature review aims to:

1. Highlight the various prevention and treatment methods currently used to address alloimmunization, DHTR, and hyper-hemolysis.
2. Evaluate each method using data from case studies, meta-analyses, surveys, and observational studies.
3. Identify gaps of knowledge in prevention and treatment.
4. Conclude with future directions and recommendations to optimally manage DHTR in SCD patients.

BACKGROUND

Pathophysiology of Red Blood Cell Alloimmunization

Antigenic differences between donor and recipient RBCs are the initial trigger of alloimmunization. RBC alloimmunization can be summarized in five steps (**Figure 1**):

1. RBC antigen recognition, processing, and presentation by human leukocyte antigen (HLA) Class II to T-cell Receptor
2. Activation of CD4 helper T cells
3. Interaction of B and T cells
4. Differentiation of B cells into plasma cells
5. Production of antibodies by plasma cells

Yazdanbakhsh et al. (2015) suggest heightened immune effector cell responses and impaired regulatory networks drive antibody production during alloimmunization in SCD patients. Altered T cell immunoregulation and higher follicular T cell responses that increase antibody production was shown in alloimmunized SCD patients. Chronically transfused SCD patients reported reduced peripheral regulatory T cell (Treg) suppression and altered T helper cells (Th). These patients also demonstrated higher inflammatory cytokine (INF-alpha) and lower anti-inflammatory cytokine (IL-10) levels (Yazdanbakhsh, 2015). These findings support a model where SCD alloimmunization is due to a generalized immune dysregulation with an imbalance between Treg and Th cells and an increase in antibody production. It is likely alloimmunized SCD patients are unable to turn off their pro-inflammatory state in response to their hemolytic state, which

increases the risk of further alloimmunization (Yazdanbaksh et al., 2017; Platt, 2000)

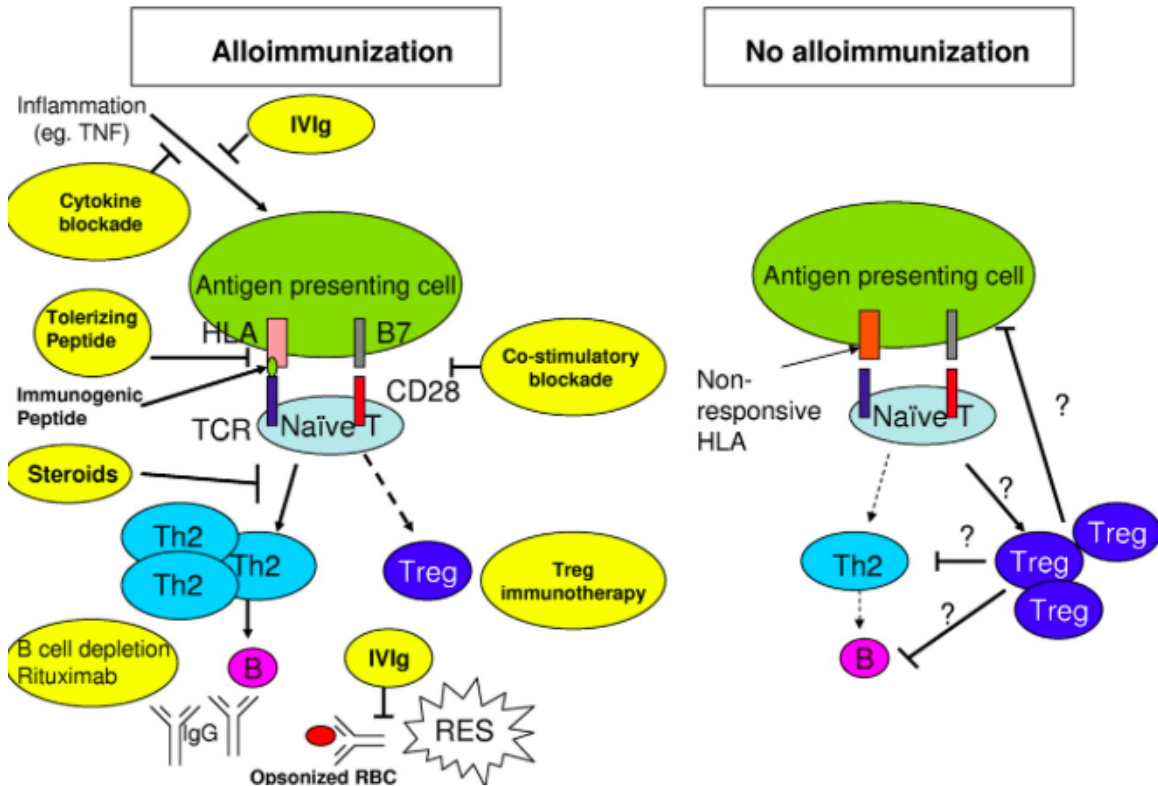


Figure 1. Immune response to RBC antigens in alloimmunized versus non-alloimmunized patients. The yellow specifies possible interventions to prevent alloimmunization and treatment of DHTR. Figure taken from (Yazdanbaksh et al., 2012).

It is important to note that not all transfused SCD patients develop antibodies, suggesting that alloimmunized patients may be a genetically distinct group with increased susceptibility to RBC sensitization. However, even within patients with inherited risks, only 30% will make antibodies, suggesting that host susceptibility factors and nature of the disease may also play a role in alloimmunization (Higgins & Sloan, 2008).

Factors Influencing Red Blood Cell Alloimmunization

Antigenic differences between donor and recipient RBCs

Racial antigenic differences play a crucial role in the increased alloimmunization in SCD patients compared with other transfused populations. **Table 1** illustrates the numerous antigenic differences between white general blood donors and recipients of predominately African descent. C, E, K, Fy_a, Jk_b, and S antigens are expressed more in whites than persons of African descent. In parallel, antibodies against these antigens are most frequently identified in SCD patients (Rosse et al., 1990).

The currently published rates of alloimmunization for SCD are between 20% to 50% in selected cohorts in US and France (Yazdanbakhsh et al., 2012). This is significantly higher than alloimmunization rates in SCD patients in Uganda (6.1%) and Jamaica (2.6%), where donors and patients are racially more similar (Natukunda et al., 2010; Olujohungbe et al., 2001). However, it can be argued that lower alloimmunization rates is due to lower usage of blood products in these countries. In thalassemia patients, a population that is also frequently transfused but are more racially similar to white donors, alloimmunization is approximately 10% (Gupta et al., 2011). In contrast, alloimmunization increased to 22% in a majority Asian thalassemia patient population (Singer et al., 2000).

Rh variants between whites and persons of African descent illustrated an additional level of complexity in matching compatible RBC units for SCD patients. Individuals of African descent have a greater genetic variation of the Rh locus and may have Rh variants that lack epitopes of normal antigen (**Table 1**). These partial Rh variants

can still cause alloimmunization to occur even when receiving Rh matched units because anti-Rh antibodies against the missing epitopes develop when transfused with donor blood carrying the normal antigen (Yazdanbakhsh et al., 2012). Studies have shown that while using Rh (D, C, E, c, e) and K matched blood, approximately 45% of chronically and 12% of episodically transfused patients still became alloimmunized (Chou et al., 2013, Silvy et al., 2014).

Increased alloimmunization also occurs when the recipient lacks the “high incidence antigen,” an antigen that is commonly expressed in almost all donor RBCs. SCD patients with rare blood types negative for Hrs, HrB, RH56, Jsb, or U are at risk for increased alloimmunization as they lack the “high incidence antigen” (Pirenne, 2003). Individuals with rare blood groups can develop antibodies against all common RBCs carrying the “high incidence antigen,” potentially leading to severe cases of DHTR (Pirenne, 2019). Lastly, it is complicated to treat SCD patients with rare blood types, as many health facilities lack the appropriate antigen-negative blood.

Table 1. Differences in blood groups between recipients and donors. All blood antigen frequencies have been obtained from The Blood Group Antigen “FactsBook.”

Category	% in white donors	% in black recipients
Common Antigens		
ABO group		
A	43	27
B	9	20
O	44	49
AB	4	4
RH		
D	88	92
C	68	27
E	29	20

c	80	96
e	98	98
KEL		
K	9	2
FY		
Fya	66	10
Fyb	83	23
JK		
Jka	77	92
Jkb	74	49
MNS		
S	51	31
s	89	93
Partial RH antigens		
Partial D among D+	1	7
Partial C Among C+	0	30
Partial e among e+	0	2
Low incidence antigens		
VS (RH20)	0.01	24-40
Sa (KEL6)	0.01	20
Rare blood groups		
U negative (MNS:-5)	0	1
Hrs negative (RH:-18)	0	0.1
HrB negative (RH:-34)	0	0.1
RN (RH:-46)	0	0.1
JsB negative (KEL:-7)	0	1

Sickle Cell Disease Specific Susceptibility Factors

SCD presents with a chronic inflammation state indicated by inflammatory cytokines (IL-1, IL-6, and IFN-gamma), increased C reactive proteins, and white blood cell (WBC) count (Hibbert et al., 2005; Jison et al., 2004). SCD can manifest into a severe clinical complication called VOC and ischemic pain (Uwaezuoke et al., 2018). VOC is indicated by increased neutrophil chemotaxis, monocyte phagocytosis, production of IL-6, and IFN-gamma (Pathare et al., 2004).

It has been previously demonstrated that viral-like inflammatory stimulus can enhance alloimmunization in murine models (Hendrickson et al., 2007). Moreover, SCD patients showed increased alloimmunization following transfusion for acute inflammatory complications (Fasano et al., 2015). Lastly, febrile transfusion reactions, an inflammatory condition where the patient's immune system attacks transfused lymphocytes, enhance alloimmunization in humans (Yazer et al., 2009). These studies support the notion that the inflammatory nature of SCD creates an altered alloimmunized potential.

Frequency of RBC alloimmunization

The published rates of alloimmunization in SCD patients are between 20% to 50%, compared with 3% to 5% in transfused patients (Balbuena-Merle & Hendrickson, 2019; Karafin et al., 2018). RBC alloimmunization prevalence can be higher than published data due to alloantibodies falling below detectable levels, lack of mandated post-transfusion antibody screening, and lack of complete medical records due to patients receiving care at different health facilities (Unni et al., 2014). Currently in the United States, there is no countrywide RBC alloantibody registry, making it difficult for hospitals to keep track of a patients' alloantibody history.

The morbidity and mortality due to RBC alloimmunization are not only understudied but also under-reported. For example, in the United States, nonfatal complications from SCD alloimmunization are not required to be reported to the FDA. The reporting of deaths due to transfusion is required in most countries. However, SCD deaths due to

transfusion complications may not be immediately and directly attributable to transfusion. Lastly, there are variations of reporting transfusion morbidities and mortalities across different countries (Balbuena-Merle & Hendrickson, 2019).

High and Low Responders

Not every transfused SCD patient makes alloantibodies in antigenic mismatch situations. Fortunately, many SCD patients receive hundreds of blood units without developing any antibodies. Thus, high and low responders can be separated in this patient population, but precise cutoffs delineating the two have not yet been identified. The prior immunological status of the patient has some predictive value. A patient who previously developed antibodies post transfusion can be considered a high responder (Pirenne, 2019).

Alloimmunization Can Lead to DHTR

Evanescence occurs when alloantibodies fall below levels of detection using traditional blood banking methodologies. Patients then can be exposed to seemingly “safe” blood containing antigens that they have been previously sensitized to. This can lead to DHTR and hyper-hemolysis, which can be particularly dangerous to SCD patients who already have endogenous RBCs with shorter circulatory half-life. The high prevalence of alloimmunization in SCD patients may be attributed to the frequency of blood transfusions a patient receives throughout their lifetime. SCD patients are usually exposed to RBCs with foreign antigens from a variety of donors (Balbuena-Merle &

Hendrickson, 2019). The result of remote antigen exposure is the development of alloantibodies in SCD patients, which is not often detected in pre-transfusion screening (Yazdanbakhsh et al., 2012). Along with the lack of detailed and longitudinal transfusion records, a SCD patient can be put at risk for alloimmunization and the resulting transfusion complications.

Alloantibodies and Autoantibodies

In the cases where antibodies are identified (approximately 70%), half are alloantibodies linked to known blood group polymorphisms (Habibi et al., 2016). In post-DHTR cases, the most frequently identified antibodies are against Fy_a, Jk_b, and S. In half of these cases, at least two antibodies were found in patients with a previous history of alloimmunizations. Furthermore, alloimmunization has also be associated with RH variant carriers (DAR, DIIIa, DIVa, and DAU), low-incidence antigens (Go_a, Js_a C_{ob}), and rare RH blood group (Hrs, Hr_B) can lead to alloimmunization (Yazdanbakhsh et al., 2012).

Autoantibodies, antibodies against self-antigens, consist of the second half (Habibi et al., 2016). Autoantibody mediated DHTR with hyperhemolysis is supported by two studies (Castellino et al., 1999; Aygun et al., 2002). In these cases, autoantibodies are hard to characterize because they appear as panagglutinins, an antibody that reacts against all reagents in an antibody panel (Yazdanbakhsh et al., 2012)

Interestingly, autoantibodies can be a risk factor in the formation of alloantibodies in chronically transfused SCD patients. In a study with over 600,000 patients across 12

hospitals, 42.2% of patients with RBC autoantibodies had at least one alloantibody (Karafin et al., 2018). In cases where autoantibodies develop during DHTR, extended matching cannot be used as a prevention method. Instead, rituximab administration before transfusion can be used to prevent alloimmunization (Pirenne et al., 2017).

Lastly, a subset of DHTR presents with no detectable antibodies. The exact mechanism of this phenomenon is still poorly understood, thus challenging to treat or prevent. It is impossible to identify patients at risk for a first or recurrent episode of DHTR.

Mechanisms of Delayed Hemolytic Transfusion Response

DHTR is a severe consequence of RBC alloimmunization. A DHTR occurs when a patient receives RBCs expressing an antigen which the patient is unknowingly sensitized against. Within four days post-transfusion, the patients produce antibodies that bind to the transfused RBCs. This leads to their destruction via phagocytosis and complement-mediated hemolysis (Pirenne et al., 2017).

DHTRs can occur between 24 hours to ten days post transfusion. It is characterized by a significant drop in Hb levels, exacerbating SCD symptoms and degree of anemia. Commonly, patients also develop severe reticulocytopenia, a decrease in immature RBCs. This requires the patient to receive additional transfusions, which may worsen their hemolytic symptoms (Pirenne et al., 2017; Yazdanbakhsh et al., 2012). DHTR can present with a wide range of symptoms, but it is difficult to predict its severity. In severe cases, the patient's own RBCs are also destroyed, a phenomenon

referred to as hyper-hemolysis. The general mechanism of hemolytic transfusion response is illustrated below in **Figure 2**.

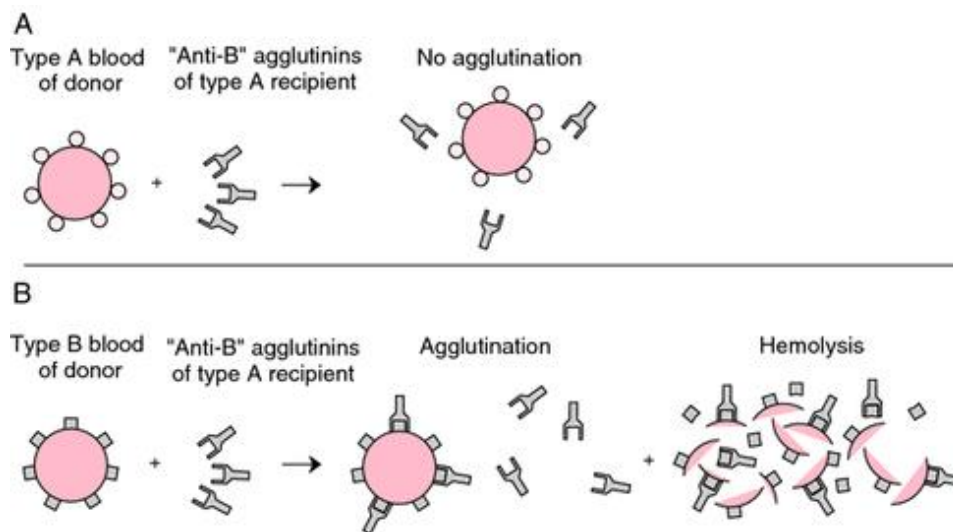


Figure 2. General mechanism of hemolytic transfusion response.

Agglutination is the process where an antibody binds to its corresponding antigen, forming a large clump of particles. Panel (B) illustrates when agglutination as a result of antigenic mismatch. Figure taken from (<https://arimmuneresponseassignment.weebly.com/report.html>)

Mechanism of Intravascular Hemolysis

Intravascular hemolysis occurs when the RBCs ruptures within the vasculature, releasing free heme and Hb into the plasma. Free Hb is vulnerable to be oxidized as it no longer has anti-oxidant sentries that are normally available in RBCs. However, free Hb can bind to haptoglobin, a plasma glycoprotein, to form the hemoglobin-haptoglobin complex. Only during hyper-hemolytic or chronic hemolysis conditions when

haptoglobin becomes depleted, free Hb is oxidized to ferric Hb and further dissociates into heme, iron, and globin (Schaer et al., 2014; Gladwin et al., 2012).

Figure 3 illustrates various ways intravascular hemolysis contributes to inflammatory injury, vasculopathy, and vaso-occlusion. Free hemoglobin drives Fenton reactions to produce oxidants. Free heme further activates the release of placenta growth factor (PGIF) and endothelin-1 (ET-1) that contribute to endothelial dysfunction, platelet activation, and pulmonary hypertension. Heme activates the production of reactive oxidative species (ROS), neutrophil extracellular traps (NET), and inflammatory cytokines, other mediators that promote the expression of adhesion receptors and ligands on endothelium and RBCs. The activated endothelium then interacts with platelets, neutrophils, and sickle RBCs to produce vaso-occlusion and acute chest syndrome (Kato et al., 2017)

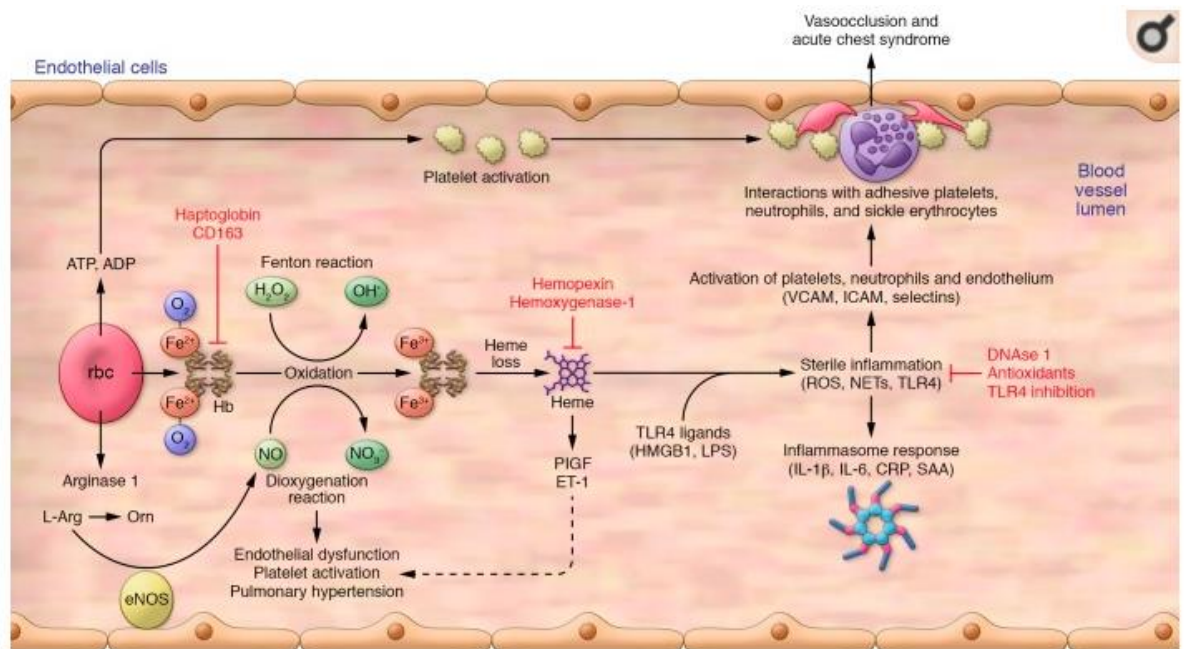


Figure 3. Contribution of intravascular hemolysis to vasculopathy and vaso-occlusion. Figure taken from (Kato et al, 2017)

Free heme can also bind to another plasma glycoprotein, hemopexin, and travel to the liver, spleen, and bone marrow to be phagocytized by macrophages during the process of extravascular hemolysis.

Complement Activation during Intravascular Hemolysis

During DHTR, intravascular hemolysis may activate the complement cascade via the classical pathway or the alternative pathway (Dumas et al., 2016). The classical pathway is activated by immune complex formation (antibody binds to RBCs), leading to C1 complex association. The alternative pathway is activated by the hydrolysis of C3 into C3(H₂O). These two pathways converge when C3 convertase converts C3 into C3a and C3b. The cascade continues to make C5b-9, also known as the membrane attack complex (MAC). The MAC destroys the targeted cell by disrupting its membrane. C3a and C5a are also anaphylatoxins that promote inflammation (Merle et al., 2019).

Figure 4 illustrates in greater detail how free heme released during intravascular hemolysis activates the alternative complement pathway. Heme promotes the transition of C3 into C3(H₂O) and the formation of fluid phase C3 convertase. This leads to C3b deposition on RBCs and their subsequent lysis. The complement cascade is further activated by amplification loop that creates more C3b, C3a, and C5b-9 (Merle et al., 2019) .

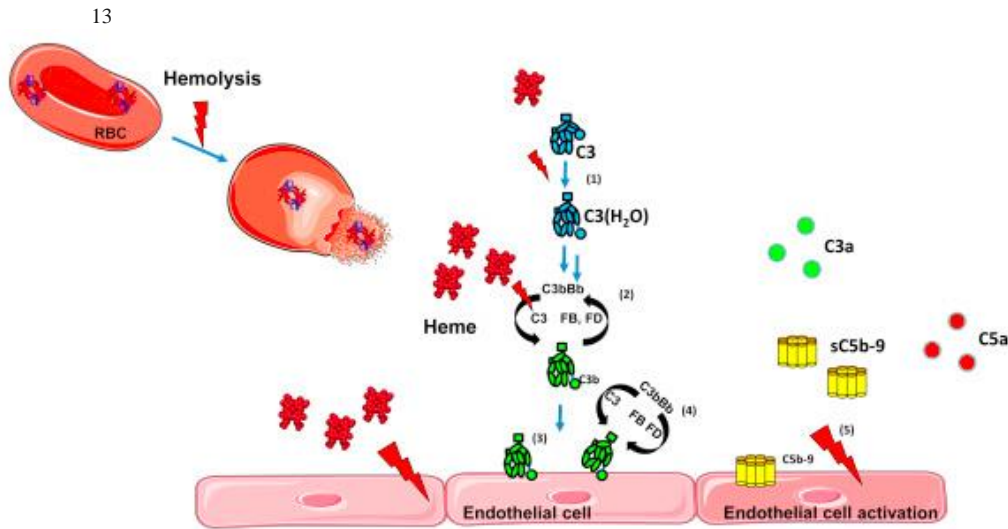


Figure 4. Free heme activates the alternate complement pathway. Figure taken from (Merle et al., 2019)

Hemolytic Parameters

Several hemolytic parameters are used in the differential diagnosis and management of hemolytic anemia. These parameters are described briefly below. The normal clinical values of each parameter are listed in **Table 2** (Barcellini & Fattizzo, 2015).

1. Hb is the most direct indicator of hemolysis. Mild forms of hemolysis can be close to normal Hb values (>10 g/dL) while severe forms are close to 6 g/dL.
2. Reticulocytes indicate bone marrow erythropoietic activity because they are non-nucleated precursors of RBCs. Reticulocyte count usually increases following hemolysis. However, an initial fall and rise in absolute reticulocyte count during recovery is common during hemolysis associated with DHTR (Win et al., 2010)
3. Lactate dehydrogenase (LDH) is an enzyme responsible for the conversion of

lactate into pyruvate. LDH-1 and LDH-2 isoenzymes are expressed specifically in RBCs. Following hemolysis, LDH levels usually increase and can be used to distinguish between intravascular versus extravascular hemolysis.

4. Haptoglobin, as described previously, is a glycoprotein with the ability to bind to free Hb to reduce the formation of reactive oxygen species and renal damage. The resulting hemoglobin-haptoglobin complexes are cleared by scavenger endothelial cells. Haptoglobin decrease following hemolysis.
5. Bilirubin is made following the catabolism of the protoporphyrin IX ring of heme. An increase in bilirubin levels may be due to increased Hb catabolism or decreased hepatic clearance. Following hemolysis, hyperbilirubinemia is usually indicated by no more than 4mg/dL. Higher values may indicate liver complications.
6. Ferritin is an intracellular protein that stores and releases iron to buffer iron deficiency and overload. It can be used to indicate the total amount of iron in a body and generally increases following hemolysis.

Table 2. Normal range of hemolytic parameters. M indicates male, F indicates female

	Normal levels
Hemoglobin	>10 g/dL
Reticulocytes	$20 \times 10^9/L$ - $100 \times 10^9/L$
Lactate Dehydrogenase (LDH)	240 - 460 U/L
Haptoglobin	41-165 mg/dL
Bilirubin	0.2 to .8 mg/dL
Ferritin	12 -300 ng/mL (M) 12-150 ng/mL (F)

Frequency and Diagnosis of DHTR

DHTR is under-reported as its clinical presentations resemble those of VOC (Fabron et al., 1999). In a retrospective study of chronically transfused SCD patients, DHTR, as evidenced by antibody detection, occurred in 30% of the cases (total 178). However, 39% of these cases were reported to blood banks as possible DHTR. 21 cases were identified as severe DTHR. (Coleman et al., 2019). DHTR mortality can be as high as 11.5% (Narbey et al., 2017)

There are no current diagnostic guidelines and consensus definition of this condition. Pirenne and Yazdanbakhsh proposed a two-step diagnosis of DHTR, based on clinical symptoms and levels of hemoglobin A (HbA) (**Figure 5**). The first step is based on vaso-occlusive symptoms (dark urine, onset, or worsening of anemia symptoms) and increasing LDH within three weeks of transfusion. This first step can indicate that DHTR is likely, allowing the physician to stop further transfusions. In the second step, DHTR is confirmed by a decrease in concentration and percentage of HbA relative to immediate post-transfusion levels. As SCD patients produce HbS and receive HbA from donor blood, lowered HbA levels signifies both the patient and donor RBCs are destroyed. The relative change in HbA concentration and days elapsed can be then used to indicate DHTR likelihood (Pirenne & Yazdanbakhsh, 2018).

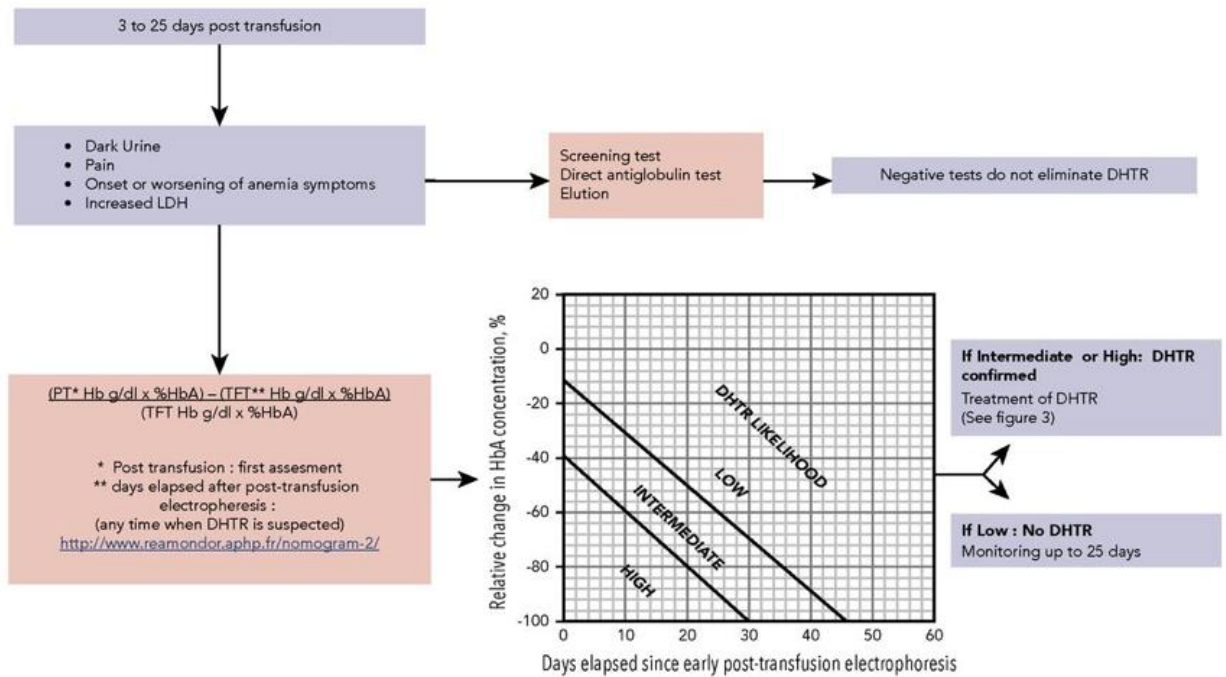


Figure 5. Diagnostic guidelines for DHTR in adult SCD patients. The assessment criteria are based on clinical and laboratory presentations, including pain, anemia, urine color, elevated LDH. Figure take from (Pirenne & Yazdanbakhsh, 2018)

As mentioned previously, a key challenge is the under-recognized incidence of DHTR, as its clinic manifestation mimics VOCs. Gardner et al. (2015) highlighted some of the key differences between the two (**Table 3**). It is important to note that a negative direct antiglobulin test (DAT) does not exclude DHTR.

Table 3. Differentiating DHTR from VOC episodes. Table adapted from (Gardner et al., 2015)

	DHTR	VOC episodes
Context	Recent RBC transfusion (within 2-21 days)	Any
Symptoms	Pain, fever, hemoglobinuria	Pain, fever
Reticulocytes	Variable- relative reticulocytopenia or elevated	Frequently elevated from baseline unless transient RBC aplasia from acute infection (e.g Parvovirus)
LDH	Highly elevated compared to baseline	Mildly elevated compared to baseline
Hb quantitation	Decrease in Hb to below post- or pre-transfusion level, rapid clearance of HbA% with concomitant increase in HbS%	Unchanged or mild decrease from baseline; or if transfused, appropriate increase in Hb
Immunohematology	DAT positive (~75%) or negative (~25%); new RBC alloantibody was detected in some cases	DAT negative

HOW CAN ALLOIMMUNIZATION BE PREVENTED?

Antigen Matching

In western countries, the standard of care for transfusing SCD patients include matching for Rh group (D, C, E, c, e) and K antigens (LaSalle-Williams et al., 2011). However, there are no standard guidelines for antigen matching beyond these antigens to prevent alloimmunization. Moreover, it is not clear which patients are likely to become alloimmunized (Yazdanbakhsh et al., 2012). Some transfusion services only provide antigen matched blood once alloantibody is detected in the patient. While some services offer extensive matching, other services only match for the most frequently immunogenic antigens (C, E, K) (Kacker et al., 2014). A multicenter study using a pediatric cohort showed alloimmunization rate dropped from 3% to 0.5% when matching for E, C, and K (Vichinsky et al., 2001).

While it is certain that antigen matching should be prioritized to prevent alloimmunization, this is no consensus on which antigens should be matched. Yazdanbakhsh recommends SCD patients to avoid exposure to highly immunological antigens (RH, KEL). For patients that are already immunized, less immunogenic blood groups (FY, JK, MNS) should be avoided.

La Salle-William et al. (2011) reviewed records for 99 SCD patients in a prophylactic extended matching program at the Colorado Sickle Cell Treatment and Research Center from the period 1993 to 2006. While most patients have homozygous SCD (HbSS), 14% had HbSC or HbS beta-thalassemia. The study population received transfusions from infancy to adulthood, with a mean range of 5 months to 19 years.

Patients and donors were phenotyped for 20 blood groups, including ABO, RH, KEL, FY, JK, Lewis, and MNS. While attempts to match each negative antigen in the recipient and donor were made for each transfusion request, a perfect match was not always possible. Mismatches were allowed for MNSs, Fyb, and Lewis due to the lower immunogenicity of these antigens. When these mismatches were allowed, 90% of transfused units were perfectly matched (LaSalle-Williams et al., 2011).

Table 4 below describes the results of this extended antigen matching program along with other patient cohorts for a wider variety of indications since 1993.

Alloimmunization, along with antibodies per 100 unit transfused, decreased with extended matching. Note that extended matching beyond ABO and D can significantly reduce the percentage of patients immunized (LaSalle-Williams et al., 2011).

Table 4. Alloimmunization in patients treated with extended matching protocol. Patients were in each group were analyzed separately. *Different from historical value, $p < 0.00005$. Table adapted from (LaSalle-Williams et al., 2011)

Patient Group	Matching	Percentage of Patients Immunized	Rate (antibodies/100 units transfused)
Chronic Transfusion n=85	ABO, D	34%	3.4
Chronic Transfusion n=12	Extended matching for all who had previously received ABO, D	25%	0.3
Chronic Transfusion n=13	Extended matching	8%*	0.08*
Chronic and Intermittent n=99	Extended matching	7%	0.10

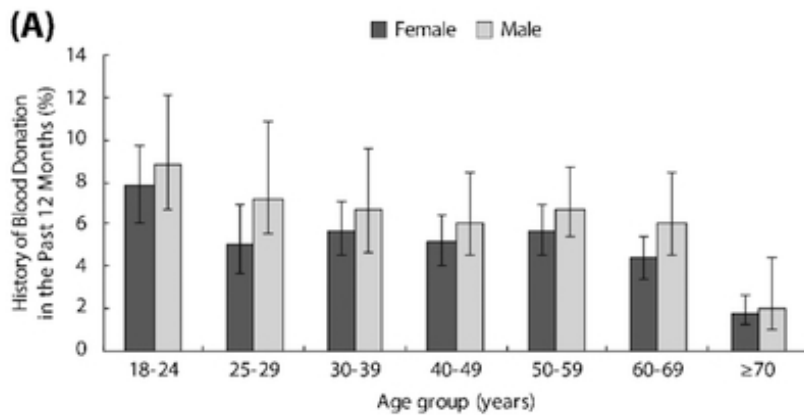
Another factor to consider is the cost-effectiveness of prospective extended antigen matching programs. The cost of medical care for one SCD patient over a lifetime is greater than \$460,000 (Kauf et al., 2009), while an estimated 700,000 to 100,000 people suffer from SCD (Hassell, 2010). Kacker et al. (2014) constructed a stimulation model (Markov-base model) to compare the health and financial implication for four different antigen-matching strategies for SCD patients over 10 and 20 year periods. The four strategies differed by the extent of antigen matching (C, E, K or C, c, E, e, K, Fy_a, Fy_b, Jk_a, Jk_b, S, s). The results showed over 10 years, prospective extended matching would cost \$1.8 billion more than history-based extensive matching while avoiding 2424 alloimmunization events. In order to prevent a single alloimmunization event, the cost would be between \$369,482 to \$769,284 (Kacker et al., 2014).

Increasing RBC donations from persons of African descent

One barrier in treating SCD patients is the lack of blood donations from minority groups. In the US, blood donation rates from AAs are significantly lower compared with whites. In 2019, a cross-sectional analysis was performed among 28, 739 participants in the 2016 National Health Interview Survey (NHIS). The NHIS is a household survey for noninstitutionalized US civilian population at both national- and census- region level. The sample includes people over 18 years old living in households and non-institutional group quarters (e.g., college) throughout the US. The survey was conducted in person by trained interviewers. The outcome of interest was determined by asking the question,

“During the past 12 months, have you donated blood?” Participants who answer “yes” were considered past year donors (Patel et al., 2019).

The prevalence of blood donations in the past 12 months stratified by age and race/ethnicity is shown in **Figure 6**. In this study, young adults were more likely to donate blood than older population. Across all age categories, a past-year history of blood donation was more common in males (6.3%) compared to females (5.1%). Across all age categories, a past-year history of blood donation was lower in AAs (3.9%) and Hispanics (3.0%) compared to whites (6.9%). Furthermore, being a college graduate, employed, physically active, and non-smoking positively correlated with donating blood. Lastly, the prevalence of blood donation was higher in the Midwest (7.4 %) and South (6%) compared with Northeast (4.7%) and West (4.4%) (Patel et al., 2019).



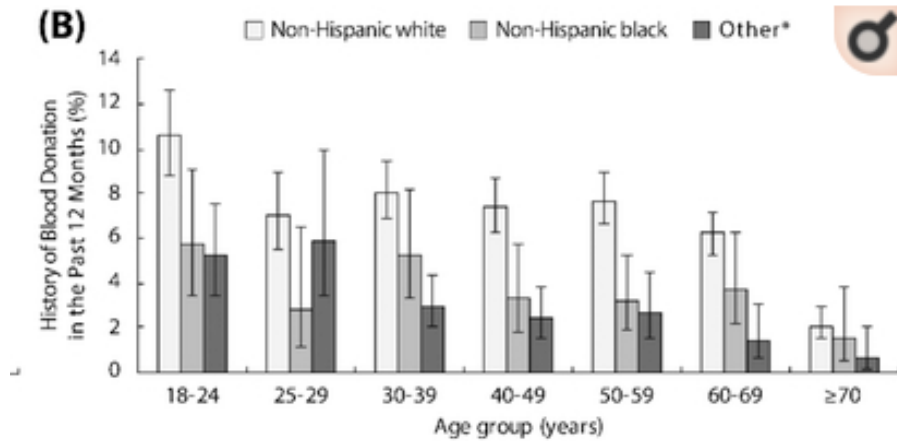


Figure 6. Age-specific self-reported prevalence of blood donation in the past 12 months. The data stratified by age (A) and ethnicity (B). “Other” consist of non-Hispanic Asian, Hispanics, and non-Hispanic other/multiracial groups. Data were weighted, and error bars reflect design-adjusted, logit-transformed 95% confidence intervals. Figures taken from (Patel et al., 2019).

The above results are consistent with a 2017 study that evaluated the changes in minority blood donation over a period from 2006 to 2015. Data from RBC donors were collected annually by eight large blood collectors in the United States. The results show that white donors donated the highest proportion of RBC units (76.3% - 80.2%) and constituted the majority of donors (76.1% - 79.8%). During the 10 year study period, AA donors contributed 4.0% to 4.3% of all donated RBC units and constituted 4.95% to 5.2% of all donors. Lastly, linear regression of the data showed a decreasing trend in the number of donors, collection, and RBC units over the study period (Yazer et al., 2017).

In 2019, Spratling et al. (2019) conducted a meta-synthesis review using various electronic databases to determine the facilitators and barriers for blood donation. Three common themes were determined (1) knowing a blood recipient, (2) identifying with

culture, race/ethnicity, and religious affiliation, (3) medical mistrust and misunderstanding. The themes are further described using examples in **Table 5** below. (Spratling & Lawrence, 2019).

Table 5. Facilitators and barriers to blood donation in minority communities. Table adapted from (Spratling & Lawrence, 2019).

Themes	Summary	Example
Knowing a blood recipient	Knowing a blood recipient (friend, direct or indirect family member) made a minority donor more likely to donate	When asked about emotions associated with donating blood, AA donors reported “hearing people’s personal stories is inspiring” and “something happens to someone in my family” (Amoyal et al., 2013).
Identifying with culture, race/ethnicity, and religious affiliation	Identifying culture, race/ethnicity, and religion provide a reason for some minority donors to donate	AA donors expressed blood donating would benefit their own community (Mathew et al., 2007) Perceived social exclusion was reported to be a fundamental issue for AA donors (Tran et al, 2013)
Medical mistrust and misunderstanding	Unclear explanation for donor deferrals and suspicion about blood donation are barriers to donation	AA donors reported feeling their blood was unwanted or their donation would be later discarded (Tran et al., 2013)

New York Blood Center (NYBC) is a nonprofit blood distribution organization that collects and processes 400,000 units of blood annually for 200 metropolitan-area hospitals. In 2005, the New York Blood center developed and implemented the PreciseMatch program to increase blood donations from AA or black and Hispanic or Latino donors to increase the chance that alloimmunized patients will receive properly

matched blood. The goal of the PreciseMatch program was to increase donations among AA and Hispanic/Latino donors by 150 incremental units per month (Frye et al., 2014).

PreciseMatch Program is a community-oriented education program that relied heavily on one-on-one outreach. Professional outreach coordinators (1) provided education in target communities to address myths and misconceptions about blood collection; (2) promoted awareness for the need of blood donations among AA or black, Hispanic or Latina, and other ethnically diverse communities; (3) built relationships with communities and their leaders. The coordinators focused their outreach at churches, community centers, health expos, parades, and street fairs in Manhattan, Bronx, and Brooklyn. The marketing materials used included formal presentations, posters, and brochures in both English and Spanish. The brochures featured three local Latino SCD patients' stories. NYBC's communication and Media Relations department also played a significant role in bringing attention to the program. Information regarding various events and blood drives were covered in all local Spanish language newspapers and radio stations with sizable AA or black audiences. A systematic analysis of program documentation and collection data revealed that PreciseMatch achieved 75% of the original goal, with 75% of donors being first time donors (Frye et al., 2014).

Rituximab

Rituximab is a chimeric monoclonal antibody against CD20, a protein found on the surface of B cells. **Figure 7** illustrates the three main pathways that rituximab depletes B cells: (1) antibody dependent cellular cytotoxicity (ADCC), (2) complement

medicated cytotoxicity and (3) apoptosis (Seyfizadeh et al., 2016). Rituximab is used generally used to certain cancers such as Non-Hodgkin’s lymphoma and chronic lymphocytic leukemia. B cell depletion using rituximab is also known to be effective against a variety of autoimmune diseases including autoantibody-mediated hemolytic anemia (Pirenne et al., 2015; Bachmeyer et al. 2010).

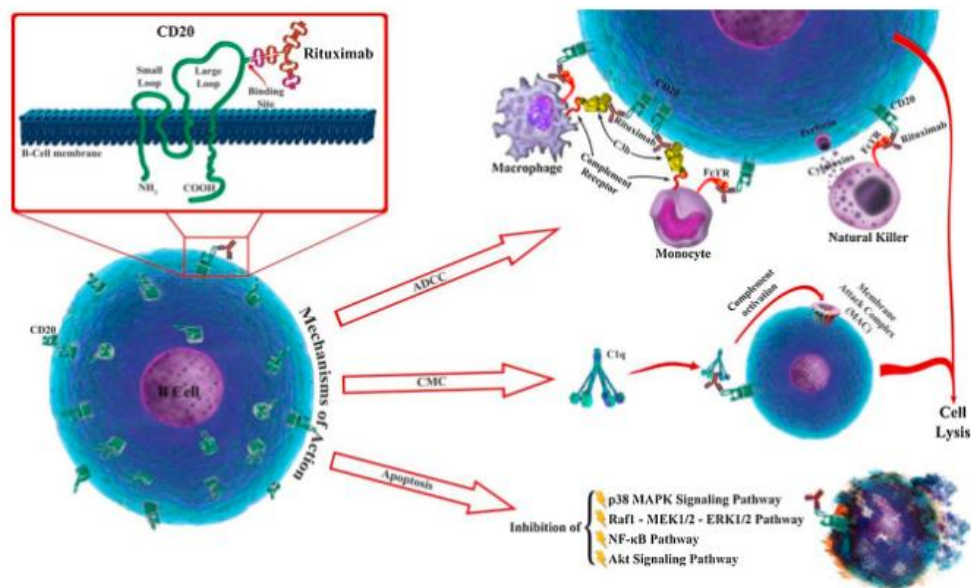


Figure 7. Mechanisms of action of rituximab to deplete B cells. Figure taken from (Seyfizadeh et al., 2016)

Several case studies have documented positive outcomes with rituximab in DHTR patients. Noizat-Pirenne et al. (2015) conducted a single-center observational study on eight SCD patients following rituximab administration and transfusion. All eight patients had a definitive diagnosis of SCD and a prior history of DHTR (one did not have a prior history but was alloimmunized). Rituximab was administered before transfusions to

prevent further immunization and DHTR. 10 mg of methylprednisolone (MP) was administered before rituximab in all cases.

All patients received units that were matched for Rh, KEL, FY, JK, and MSN blood groups. Following transfusion, five patients showed good clinical outcome defined by the lack of post transfusion VOC, lack of hemoglobinuria, and HbA that persisted within 1 month post transfusion. The remaining three patients presented mild DHTR defined by a rapid decrease of HbA and total Hb within 5 to 15 days post transfusion. One patient also presented with mild vaso-occlusive complications. Lastly, the results of post transfusion and pre transfusion screening test exactly matched in all eight patients, indicating that no new antibodies were detected (Noizat-Pirenne et al., 2015).

Pirenne et al. (2015) demonstrated that rituximab could prevent the formation of new antibodies and minimize the risk of severe DHTR. In cases when a patient with prior DHTR history and needs additional transfusions, rituximab can be considered. However, long term usage of rituximab should be evaluated as chronic B cell depletion can increase the risk of infection, especially those with functional asplenia. The patient should be vaccinated against pneumonia at least two weeks before using rituximab. Furthermore, the three patients who still developed mild DHTR in the absence of newly antibodies suggest that DHTR depends on other complex heterogeneous mechanisms in addition to the classical antibody-mediated responses (Pirenne et al., 2015).

Nickel et al reported a case where the use of prophylactic rituximab in a SCD patient with previous DHTR history had a fatal outcome. The patient (19 yo, male)

desired a hip replacement surgery due to daily pain and ambulatory difficulties. He previously suffered three DHTRs, hyper-hemolysis, and had a history of multiple RBC alloantibodies (anti-C, -E, -K, -Fy_a, -Fy_b, -Jk_b, and -M) and autoantibodies. Despite using rituximab preemptively to prevent DHTR, the patient still suffered from severe DHTR, hyper-hemolysis, and Salmonella sepsis 3 days post transfusion. The patient passed away three days later (Nickel et al., 2016).

HOW IS DHTR CURRENTLY TREATED?

Corticosteroids

High dosage corticosteroids have been used as the first line of defense for severe DHTR cases, commonly with intravenous immunoglobins (IVIg) (Vidler et al., 2015). Corticosteroids are known to improve antibody-mediated hemolysis, pain episodes, acute chest syndrome, and other steroid-responsive conditions. However, studies indicate that corticosteroids are associated with severe vaso-occlusive complications. Darbari et al. (2008) reported four SCD patients with vaso-occlusive events after administration of systemic steroid therapy. Other similar cases were also reviewed from literature to determine the potential risks associated with systemic corticosteroids. Some of the adverse health effects are present below in **Table 6** (Darbari et al., 2008).

Table 6. Adverse health effects of steroids use in SCD patients. Table adapted from (Darbari et al., 2008). ACS = acute chest syndrome. VOC = vaso-occlusive crisis

Age of Patient	Time to Adverse outcome	Complications	Author's Conclusion	Citation
21	Few days	Pain crisis, mental confusion, cardio-respiratory arrest followed by death; evidence of bone marrow necrosis and fat embolization on autopsy	Elevated level of IgG and treatment with corticosteroids appeared to a major factor in the death	(Shapiro & Hayes, 1984)
25	3 days	Pain crisis, respiratory arrest, followed by death; evidence of fat embolism, bone marrow necrosis on autopsy		(Johnson, Stastny, & Rucknagel, 1994)

3-18	Within 14 days	Hemorrhagic stroke (9)	Primary hemorrhage in children with SCD is associate with recent transfusion of corticosteroid	(Strouse et al, 2006)
7- 46	3-45 days	Bone marrow infarction (3), fat embolism (1), ACS (3), VOC (3), priapism(1), neurological symptoms (1)	Use of system steroid can precipitate severe vaso-occlusive episodes in SCD patients	(Darbari et al., 2008)

Darbari et al. (2008) suggest a causative role of corticosteroids in the presented detrimental health events due to three main observations: (1) health events followed corticosteroid administration, (2) vaso-occlusive episodes become more severe compared with pre-corticosteroid clinical course, (3) detrimental health events reoccurred in some patients following re-initiation of systemic corticosteroid therapy (Darbari et al., 2008). Although the exact mechanism of how corticosteroids induce vaso-inclusion is unclear, it should be used minimally, especially in children. Elenga et al. (2008) reported two children presenting with severe neurological complications in children after receiving corticosteroid therapy. Garden et al. (2015) recommends 2 mg/kg/day of MP with a maximum dosage of 60 mg/d along with a slow tapering period in children (Elenga et al., 2008; Gardener et al., 2015).

Intravenous Immunoglobulin

Immunoglobulin therapy is used to treat a variety of health conditions, and its

usage is expanding in autoimmune diseases (Katz et al., 2007). IVIg is frequently used to treat DHTR even in cases where no antibodies were detected (Pirenne & Yazdan bakhsh, 2018). In a retrospective case review in France, Montalembert et al. (2011) studied eight pediatric SCD patients that developed DHTR for the period 2006 to 2009. Four patients that received IVIg therapy (1-4 injections, 0.5 -1 g/kg) showed success in infusion tolerance and resolution of the hemolytic process. Two patients without detectable antibodies were also treated successfully with IVIg. Pirenne and Yazdanbaksh recommend IVIg as the first line of defense for DHTR patients with post transfusion hemolysis regardless of antibody detection, because antibodies can be formed at a later point (Pirenne & Yazdanbakhsh, 2018).

A case study published by Win et al. (2010) further supports the use of IVIg therapy. A patient (35 yo, female) presented with severe DHTR and a previous history of alloantibodies against S, Fy₃, and Jk_b (**Figure 8**). Two days following admission, the patient's Hb dropped to 47g/L. The patient was administered IVIg (0.4 g/kg) for 5 days and MP (500mg/d) for 2 days. The patient's Hb level, absolute reticulocyte count, bilirubin, and LDH level is shown below with the administration of IVIg and MP. The patient was discharged 11 days later with Hb level of 65 g/L. Furthermore, Win et al. (2010) identified five more patient cases where anemia and hemolysis were resolved using IVIg and MP in the absence of additional transfusions (Win et al., 2010) .

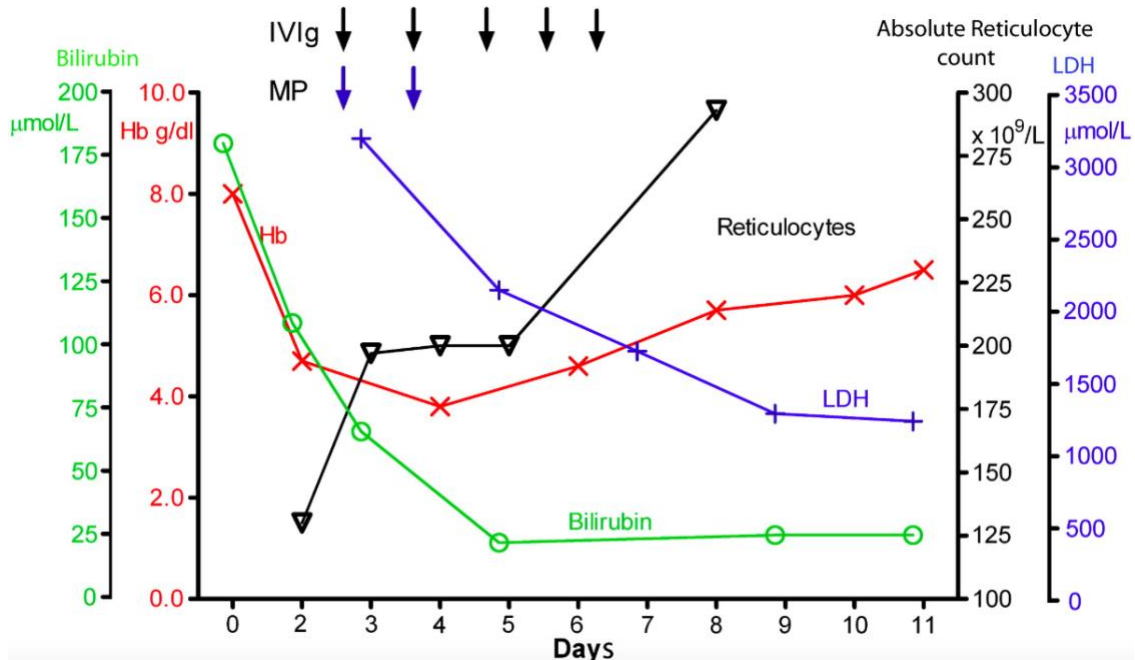


Figure 8. Hb concentration, absolute reticulocyte count, bilirubin, and LDH after treatment with IVIg and methylprednisolone. Arrows indicate when IVIg or MP were administered. Figure taken from (Win et al., 2010).

The mechanism of action of IVIg in alleviating DHTR symptoms remains unclear. IVIg therapy may act by inhibiting the cellular immune response associated with SCD (Montalembert et al., 2011). For example, IVIg can inhibit inflammation and macrophages by inducing the production of interleukin-1 receptor antagonist (Crow et al., 2007). Also, in SCD mouse models, it was demonstrated that IVIg inhibited neutrophil adhesion to reverse vaso-occlusive crisis (Chang et al., 2008).

In a literature review with over 200 patients receiving IVIg for different autoimmune diseases, 24% to 36% reported adverse effects after a high dosage. The majority of these events were immediate and mild. These included headaches, face flushing, malaise, fever, chills, fatigue, dyspnea, nausea, vomiting, diarrhea, changes in

blood pressure, and tachycardia. Later adverse health events tend to be more serious. These include acute renal failure, thromboembolic events, skin reactions, and autoimmune hemolytic anemia. However, the authors concluded that IVIg is a safe therapy to use if infused slowly in well hydrated patients and avoiding patients with known risk factors (Katz et al., 2007).

Eculizumab

Eculizumab is a monoclonal antibody that inhibits C5 convertase by preventing the cleavage of C5 into C5a and C5b (Pirenne et al., 2017). By stopping the complement cascade at this stage, terminal complement activation is inhibited. Eculizumab is used to treat atypical uremic syndrome and paroxysmal nocturnal hemoglobinuria (PNH). Eculizumab decreases the destruction of RBCs and increases transfusion independence in PNH patients (Martí-Carvajal et al., 2014).

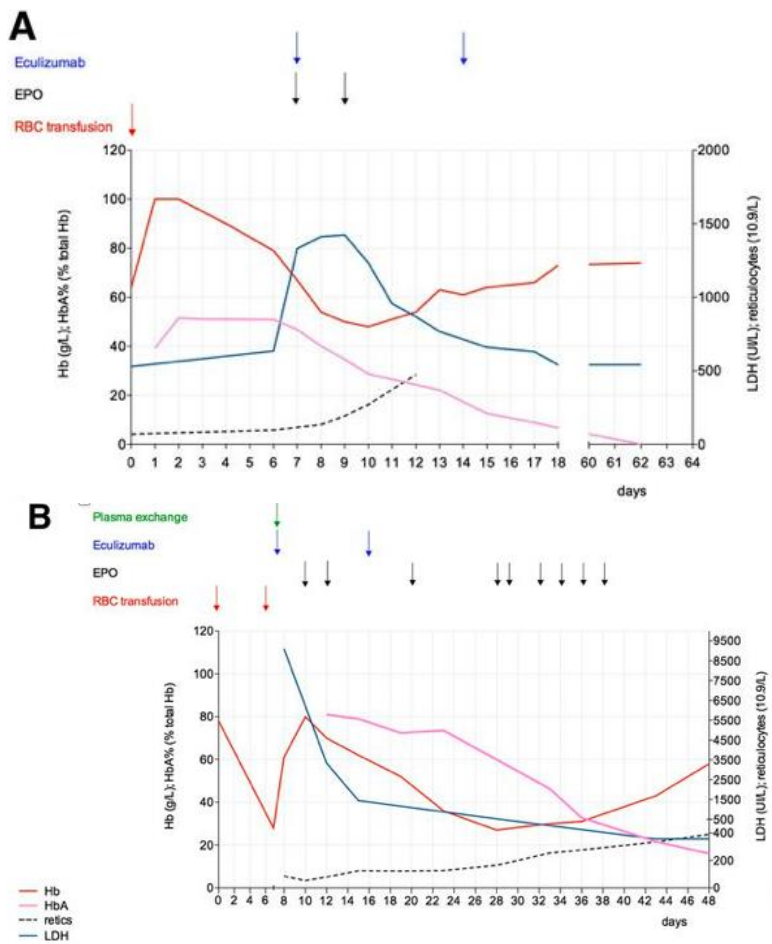
The efficacy of eculizumab in treating DHTR was analyzed in a case series by Dumas et al. (2016) at the French Sickle Cell Referral Center. This study reported data from 3 homozygous SCD patients hospitalized for DHTR with hyper-hemolysis. All three patients presented without detectable antibodies following transfusion. Two fixed doses of eculizumab (900 mg) were administered to each patient one week apart as salvage therapy. In addition, plasma was collected and frozen from each patient to characterize complement activity. **Figure 9** illustrates the event story and treatment for all three patients (Dumas et al., 2016).

Patient 1 (20 yo, female) presented with severe acute VOC 6 days post transfusion (**Figure 9A**). The transfused units were matched with Rh, Jell, Fy, and MNS blood groups due to a previous history of anti-S antibody production. DHTR was diagnosed based on the decreases in Hb and HbA. After the first day of eculizumab administration along with erythropoietin (EPO), the patient's bone pain disappeared and her urine became yellow. Hemolysis, as indicated by haptoglobin and bilirubin levels, gradually decreased while Hb levels increased. The patient was discharged on day 18. (Dumas et al., 2016).

Patient 2 (17 yo, female) presented with severe acute chest syndrome, fever, and dark-color urine 7 days post transfusion (**Figure 9B**). Because the patient had severe anemia and acute kidney injury, two units of RBC cross-matched for Kell and Rh was given to the patient. The patient's clinical condition deteriorated quickly, with acute hemolysis, respiratory, liver, cardiac, and kidney failure. Transfusion was immediately stopped, and plasma exchange was administered along with eculizumab and EPO. Gradually, intravascular hemolysis, kidney, respiratory, cardiac, and liver abnormalities were all resolved. Hb levels also increased, and the patient was discharged on day 48 (Dumas et al., 2016).

Patient 3 (18 yo, male) presented with VOC and dark-colored urine 7 days post transfusion (**Table 9C**). He received units that were matched for D, C, E, and Kell. On day 9, the patient's condition deteriorated with shock, acute liver failure, kidney injury, and increase in hemolytic parameters. Eculizumab, broad-spectrum antibiotics, platelet, and plasma transfusions were administered. On day 13, the patient received a liver

transplant. Due to this major surgery and the large number of blood units needed, the patient received a total of 59 RBC units matched only for Rh and Kell during day 8 to 22. The patient's haptoglobin level increased to 0.42g/L on day 10, indicating the disappearance of hemolysis. Unfortunately, the patient died on day 23 from a severe pulmonary infection (Dumas et al., 2016).



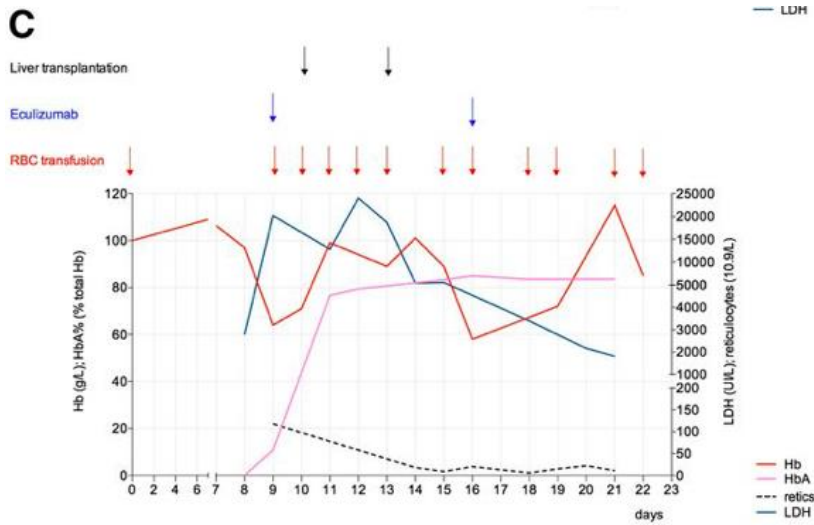


Figure 9. Hb, HbA, reticulocytes, and LDH after treatment of eculizumab. Time history of patient 1 (A), patient 2 (B), patient 3(C). Day 0 indicates the day of the transfusion responsible for the hemolytic episode. Blue arrows represent eculizumab infusion. Black arrows indicate EPO injection. Red arrows indicate RBC transfusion days. Green arrows represent plasma exchange sessions. HbA is indicated as a percentage of total Hb concentration. Figure taken from (Dumas et al., 2016)

Table 7 summarizes the complement data before and after eculizumab administration. The high level of soluble terminal protein complex (sC5B9) indicates MAC formation and terminal complement activation occurred in all three patients. **Table 8** summarizes the normal values of C3, C4 and sC5B9 (Dumas et al., 2016).

Table 7. Complement Analysis Before and After Eculizumab Infusion. Data is in mg/L *Day after first eculizumab injection. Table adapted from (Dumas et al., 2016)

	Day*	C3 level	C4 level	sC5B9 level
Patient 1				
Before eculizumab infusion	0	1080	266	856
1 st follow up	12	1030	283	1233
2 nd follow up	14	1050	248	1406
Patient 2				
Before eculizumab infusion	0	-	-	-

1st follow up	7	1270	257	1797
Patient 3				
Before eculizumab infusion	0	1070	220	1527
1st follow up	4	313	104	296
2nd follow up	6	359	129	224
3rd follow up	9	928	526	355
4th follow up	13	1210	290	509

Table 8. Normal Values of C3, C4, and sC5B9. Table adapted from (Dumas et al., 2016)

C3	660-1250 mg/L
C4	93-380 mg/L
sC5B9	<450 ng/mL

These data exemplify the potential severity of DHTR with hyper-hemolysis.

Dumas et al. (2016) suggest that eculizumab should be administered at the first sign of DHTR. However, using eculizumab carries the risk of infection with encapsulated organisms, such as meningococcal disease. Meningococcal vaccination is recommended at least two weeks before receiving eculizumab (Benamu & Montoya, 2016). In 2017, the CDC released a report indicating that there were 16 cases of meningococcal disease in eculizumab recipients from 2008 to 2016. The majority of these cases occurred in recipients with at least one dose of meningococcal vaccine before disease onset, indicating that prophylactic vaccination can-not be expected to prevent all cases of infection (McNamara, 2017).

Tocilizumab

Tocilizumab is a monoclonal antibody against the interleukin-6 receptor (IL-6). It is commonly used to treat rheumatoid arthritis, systemic juvenile idiopathic arthritis,

giant cell arteritis, and macrophage activation syndrome (Sivapalaratnam et al., 2019). IL-6 is an inflammatory cytokine that also induces B cell and T helper cell differentiation. When secreted by neutrophils and macrophages, IL-6 induces endothelial cells that release chemokines to recruit more immune cells and acute-phase proteins. When tocilizumab binds to IL-6R, the pro-inflammatory functions of IL-6 are inhibited (Sheppard et al., 2017).

Sivapalaratnam et al. (2019) reported successful treatment using tocilizumab in a SCD patient suffering from DHTR with hemolysis. The patient (33 yo, male) presented with pain crisis, respiratory failure, and ACS with a previous history of DHTRs (**Figure 10**). He was immediately given an emergency automated red cell exchange transfusion (ARCET), treated with IVIg (0.4 g/kg/day) for 5 days, and MP (500 mg) for 3 days. Despite this, his Hb dropped sharply while hemolytic markers (LDH, bilirubin, ferritin) increased. He was treated with tocilizumab (8 mg/kg) intravenously for two days. Two days after tocilizumab treatment, the patient's urine cleared, reticulocyte increased, and hemolytic markers improved. Alloantibodies and autoantibodies were not detected in his serum screening. The patient tolerated tocilizumab well as no adverse effects were reported (Sivapalaratnam et al., 2019). Lee et al. (2019) reported a similar case where tocilizumab along with hemoglobin-based oxygen carrier-201 (a bovine based blood substitute) drastically resolved hemolysis in a SCD patient (Lee et al., 2019).

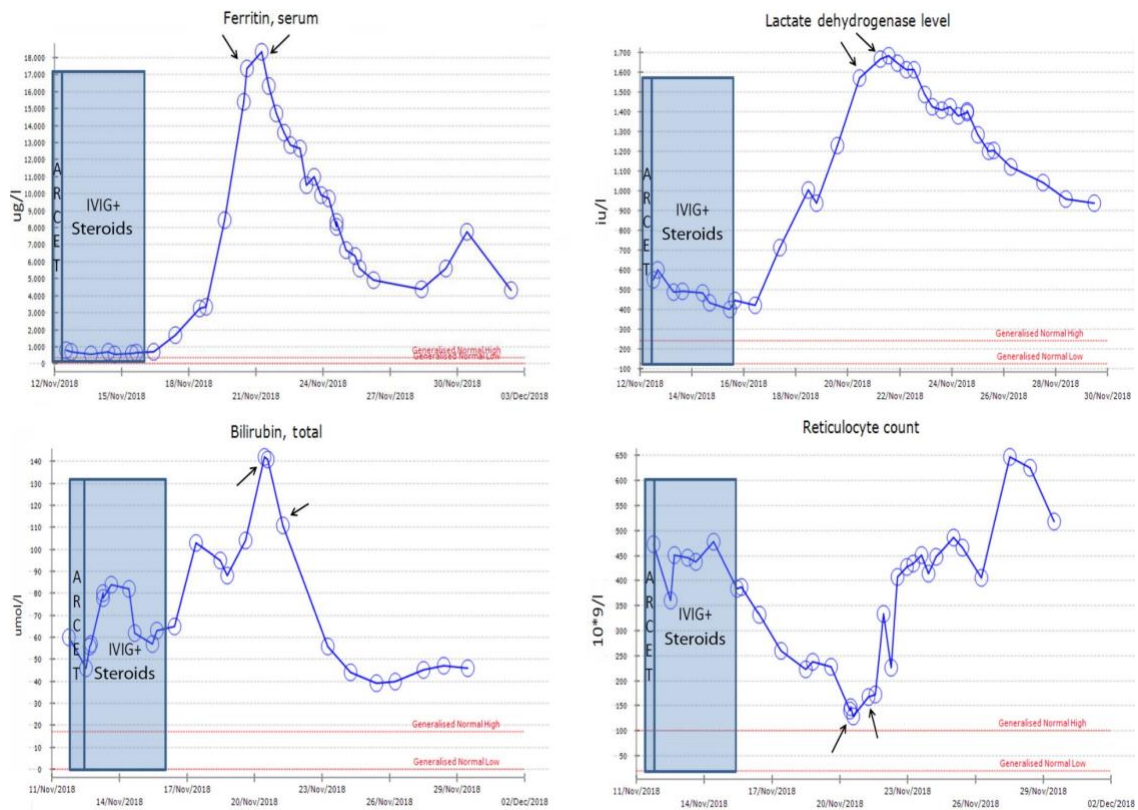


Figure 10. Serum ferritin, LDH, bilirubin, and reticulocyte count after two doses of tocilizumab. Black arrows indicate tocilizumab administration. Figure taken from (Sivapalaratnam et al., 2019).

The most common adverse effects associated with tocilizumab in rheumatoid arthritis patients are upper respiratory tract and gastrointestinal disorders. Respiratory tract infections include the common cold, pneumonia, and staphylococcus cellulitis. Gastrointestinal disorders include abdominal pain, mouth ulceration, and gastritis (Sheppard et al., 2017).

An alternative to transfusion and immunosuppressants: Hematopoietic Stem Cell Transplantation

An alternative to transfusion therapy for SCD patients is hematopoietic stem cell transplantation (HSCT). Despite HSCT being a curative therapy, its use remains limited due to lack of donor options, perceived mortality in procedures, referral bias, provider preference, poor education and families, and costs. While both the long-term and disease-free survival exceed 90% for matched sibling donor HSCT in children, the impact on adults remains largely unknown. In addition to the limited number of clinical trials in adult SCD patients, there is a general lack of consideration and popularity for HSCT as therapy for adults (Aslam et al., 2018).

The under-utilization of HSCT can be partly explained by the lack of donor availability. When patients do not have a suitable related HLA-matched donor, other potential sources of donors include adult volunteers and banked umbilical cord blood units. Using human HLA data from the National Marrow Donor Program and cord-blood-unit registry, Gragert et al. (2014) built population based genetic models to predict the likelihood of finding a suitable donor in the US registry for 21 US racial and ethnic groups. Their data suggested that most HSCT candidates will find a suitable adult donor (HLA- matched or minimally mismatched). However, many patients will not find an optimal adult donor that is matched at high resolution at HLA-A, HLA-B, HLA-C, and HLA-DRB1. The likelihood of finding an adult donor also varies within racial and ethnic groups, with White Europeans having the highest probability (75%) and AA (19%), Black (18%), South or Central American (16%), Black Caribbean (19%) having the

lowest (Gragert et al., 2014). Other studies have reported that only 14%-35% of SCD patients have an HLA-matched related sibling (Stallings et al., 2019).

Interestingly, sociocultural factors in both patients and providers may also contribute to the under-utilization of HSCT in both adults and children (Bakshi et al., 2017). Stallings et al. (2019) used a nation-wide web-based survey of pediatric hematologists/oncologists to identify the clinical attitudes surrounding HSCT. The 37 question survey was conducted for the period February to May 2016 and distributed to 70 pediatric hematology/oncology fellowship programs across the US. Survey responses were analyzed using multivariable linear regression to determine the relationship between clinician attitude and practice of discussing this therapy with SCD patient families (**Table 9**) (Stallings et al., 2019).

Table 9. Clinician attitudes about hematopoietic stem cell transplant as therapy for SCD in children. Graft versus host disease (GVHD). Table adapted from (Stallings et al., 2019).

	Percentage	95% CI
Adequacy of evidence and comfort discussing HSCT		
Reported that they felt comfortable discussing HSCT	92%	88-95
Believed there is adequate evidence to support HSCT	93%	90-96
Safety and Efficacy of HSCT		
The mortality rate after HSCT is too high to justify its use in most children with SCD	69% disagreed	63-74
The rate of cure after HSCT is too low to justify its use in most children with SCD	77% disagreed	71-81
The risk of GVHD* after HSCT is too high to justify its use in most children with SCD	70% disagreed	64-75
Perception about the cost of HCT		

The cost of HSCT to the family is too high to justify its use in most children with SCD	12 % agreed	9-16
The cost of HSCT to the system is too high to justify its use in most children with SCD	11% agreed	8-15

Figure 11 and **12** below illustrates the relationship of clinician attitudes and the timing of discussing of HSCT with patient families. Clinicians with more negative views about the safety and efficacy of HSCT were more like to discuss HSCT after the patient passes a certain threshold of disease. Similarly, clinicians who reported high costs were more likely to discuss HSCT after the patient passes a certain threshold of disease (Stallings et al., 2019).

In 2014, an international consensus recommended all SCD patients with symptomatic disease and an available donor undergo HSCT as soon as possible. However, the results of this study reported nearly half of clinicians used their own perspectives about what constitutes “diseases severity” to guide their timing of discussing HSCT (Stallings et al., 2019).

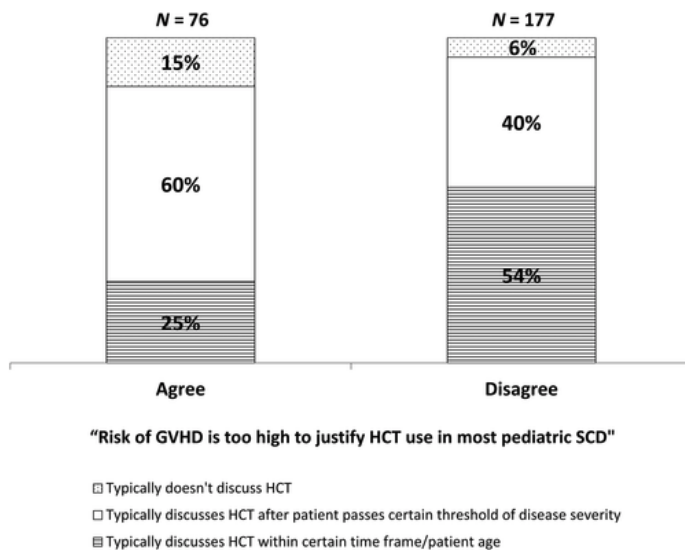


Figure 11. Relationship between clinician attitudes about GVHD and timing of discussing HSCT with patient families. Figure taken from (Stallings et al., 2019).

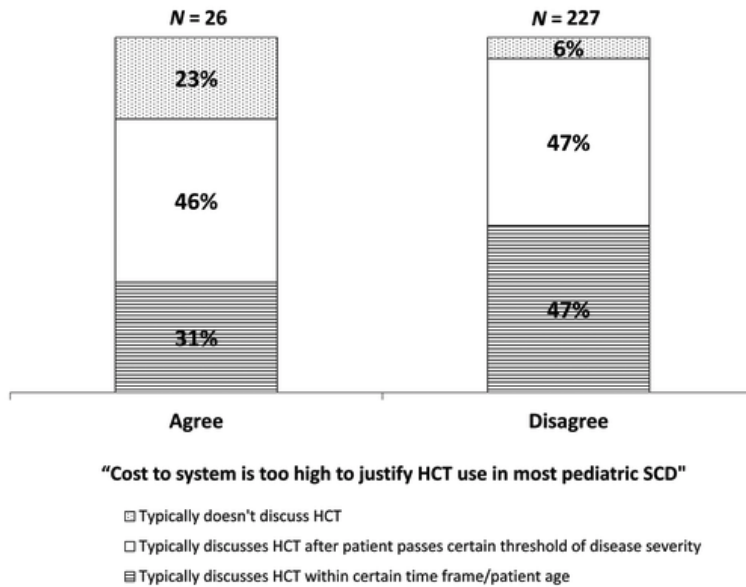


Figure 12. Relationship between clinician attitudes about the cost of HSCT and timing of discussing HSCT with patient families. Figure take from (Stallings et al., 2019).

Lastly, approximately two-thirds of providers reported treatment adherence as a decision factor in informing a family about HSCT (Stallings et al., 2019). Low socioeconomic status (SES) patients/families have many barriers to treatment adherence, including, low health literacy, housing or food insecurity, and difficulty in securing transportation. Adherence to HSCT therapy may be especially challenging to low SES patients/families as it requires a multitude of appointments, tests, imaging, and vaccinations (Walsh et al., 2014).

EVALUATION OF METHODS

Current projections predict that the number of newborn babies with SCD will exceed 400,000 by 2050 (Aslam et al., 2018). While transfusions remain the cornerstone of SCD therapy, frequently transfused patients are at risk of alloimmunization, DHTR, and resulting life-threatening complications. In this section, the current methods in preventing alloimmunization and treating DHTR are evaluated to identify gaps of knowledge and future directions.

Antigen matching to reduce alloimmunization

LaSalle- William et al. (2011) showed the effectiveness of prophylactic matching for Rh, KEL, FY, JK, MSN antigens. SCD patients typically have the phenotype D+C-E-C+e . To avoid alloimmunization against C and E, SCD patients are typically given units with the same phenotype, which is found in less than 2% of white donors. The alternative is to use units with D-C-E-C+e+ phenotype, which will deplete the already limited D- units. However, white donors who are D- typically express other immunogenic antigens (Fy_a, Jk_b, S). The use of D- units from white donors can thus expose black recipients to these antigens that lead to alloimmunization (Yazdanbakhsh et al., 2012).

Moreover, prospective matching does not benefit every patient as Rh-compatible units do not totally prevent alloimmunization due to numerous Rh variants that exist in SCD patients (**Table 1**). Lastly, the mechanism of action for antibody negative DHTR is still unknown. It is possible that DHTR may act through a non-antibody mediated

pathway. In combination with its high cost (Kacker et al., 2014) prospective matching for all transfused patients may not be an optimal strategy.

Increasing blood donations from African Americans and other minorities

Strategies to increase RBC donations from AA and other minorities can significantly increase RBC shortage for SCD patients and decrease alloimmunization. The distribution of blood donors remains mostly unchanged over the past 10 years, with continued under-representation of AA, Hispanic, and Asian donors. Sprawling and Lawrence identified knowing a blood recipient, identifying with a culture, race/ethnicity, and religious affiliations as facilitators for blood donation. Sprawling, Lawrence, and Lyre et al all identified medical mistrust as the primary barrier in blood donations. There is clear support that strategies should focus on building rapport and establishing good relations, as social, cultural identity, and trust play a significant role in blood donations among minority groups.

The PreciseMatch is one example of a successful program to increase blood donation from minority groups. However, the program was difficult to implement as it takes a considerable amount of time to establish good relationship with the relevant population. The activities to build rapport and educate members took more time and energy than anticipated, resulting in fewer blood drives and blood collections. Maintaining drives and collections requires sustained manpower and financial support. Currently, the PreciseMatch program is not as robust due to market pressures and lack of funding (Frye et al., 2014).

One interesting challenge to highlight is the higher deferral rate in AA women (25%) compared with white women (75%), due to lower normal Hb concentration among AA women (Shaz et al., 2010). Some AA women donors reported believing that deferral due to low Hb prevented them from ever donating blood (Frye et al., 2014).

Lastly, there is an additional concern with increasing RBCs units from AA donors. Up to 10% of AA will have the sickle cell trait (SCT) and carry one gene for sickle Hb. While people who carry SCT lead normal lives without any medical complications, it is not known whether transfusing SCD patients with SCT blood products will cause any adverse side effects. In the US, persons with SCT are eligible to donate blood. However, transfusion guidelines for SCD recommend the leukoreduction of RBC units by filtration. In many cases, blood from SCT donors do not filter adequately (Yazdanbakhsh et al., 2012).

Immunosuppressants to treat DHTR

The current treatment of DHTR is quite limited, with a focus on the five immunosuppressive agents listed so far this literature review. The various are summarized and compared in **Table 10** below. The various agents are listed in decreasing documented usage, with tocilizumab being used the most recently as a treatment for DHTR.

Table 10. Summary of immunosuppressive drugs for DHTR.

	Corticosteroids	IVIg	Rituximab	Eculizumab	Tocilizumab
Mechanism of Action	Unclear but possible suppression of macrophage activity	Not completely understood	Depletes B cells	Inhibit terminal complement pathway	Inhibits IL-6R
Adverse health effects	VOC, hemorrhagic stroke, respiratory complications	Typically mild, Severe effects include renal failure, hyperviscosity, and hemolysis	Increase risk of infection after chronic use	Increased risk of infection after chronic use	Upper respiratory tract and gastrointestinal disorders, but not documented in DHTR patients
Vaccination Recommended?	No	No	Yes	Yes	No
Fatal outcome in DHTR	Yes	Yes	Yes	Yes	No

IVIg and corticosteroids are currently the standards of care. However, there are no guidelines for using them as a first-line treatment. There are also no evidence-based studies to support the use of one over the other. The risks of VOE aggravation, hyperviscosity, and kidney toxicity associated with corticosteroid and IVIg usage are more severe compared to the side effects of rituximab, eculizumab, and tocilizumab. Their use should be carefully evaluated for each patient, especially in children.

While eculizumab and tocilizumab are used to treat DHRT, rituximab is used to prevent new antibody formation if the patient requires additional transfusions. Prolonged use of rituximab and eculizumab increases the risk of infections; their use should be carefully evaluated in immunocompromised SCD patients. The lack of adverse health effects in tocilizumab can be due to the small number of case studies documenting its

usage. Lastly, it is also important to take into consideration the cost of these drugs. Eculizumab is considered to be one of the most expensive drug in the world, averaging about \$18,000 per dose or approximately \$500,000 annually (“Why is Soliris the Most Expensive Drug in the US?,” 2017).

The highlighted case studies in this review also demonstrate the potential complexity and severity in DHTR cases. The progression from mild to severe DHTR is largely unpredictable. In cases of severe DHTR, a patient’s immune activation and response to treatment may also differ. Additional mechanistic studies to understand the pathophysiology of alloimmunization and DHTR are essential to provide tailored treatment for SCD patients.

While HSTC is a viable alternative to transfusion therapy, its utilization currently remains low in the US due to limited donors, perceived mortality in patients, and clinician attitudes. Stalling et al. (2019) demonstrated that pediatric hematologist/oncologists hesitate in discussing HSCT as a treatment option with patient families due to concerns with safety, efficacy, cost and treatment adherence.

Sickle Cell Disease as a Social Disparity

This literature review has shown that treatment for DHTR in SCD is largely insufficient. There are currently no clinical trials or new drugs that specifically treat DHTR. The aforementioned immunosuppressants are drugs frequently used to treat other immune disorders. This review also found frequent recurrences of authors in this topic, suggesting that few studies are done on this much-needed area of research. Moreover, a

majority of the literature on SCD and DHTR is published in journals relating to transfusion and blood disorders. It is equally important that issues surrounding SCD and DHTR be analyzed from medical, public health, and health policy perspectives.

The low public interest and knowledge in SCD can also be attributed to structural violence. Structural violence refers to how society's structure, systems, and policies unjustly affect the well-being of individuals. While the reasons for the absence of effective treatment options is multifactorial and difficult to pinpoint, some argue it is because such endeavors are not seen as financially or politically profitable (Shiffman, 2006). Bhar and Song argue health disparities faced by SCD patient in the US are a result of economic and racially motivated factors that make up the rubric of structural violence (Bahr & Song, 2015).

Bahr and Song compared the funding of SCD with cystic fibrosis (CF), another genetic disorder that primarily affects whites. In 2012, NIH reported spending \$65 million on SCD compared to \$86 million on CF, a disease with one third of the prevalence of SCD. Analysis of private funding from philanthropic support and grant income revealed that the Sickle Cell Disease Association of America received \$905,835 while the Cystic Fibrosis Foundation received \$134,090,038 in 2012 (Bahr & Song, 2015).

Race must enter this conversation, as it is people of African descent and low SES that are most commonly affected by SCD worldwide (Palermo et al., 2008). Racial disparities in the quality of care in many types of cancers, HIV, and cardiac disease for AA have been extensively documented in the US (Bahr & Song, 2015). It is possible that

racial bias, intentional or unintentional, plays a factor in the lack of new therapies and funding for SCD.

The low SES status of most SCD patients may contribute to the lack of funding and interest. SCD patients are generally poor, uninsured, and less educated.

Pharmaceutical companies with strong financial ties to their stakeholders may not want to pursue treatment that is not profitable. With less wealth and education, SCD patients also face barriers in reaching those in power and of public opinion (Bahr & Song, 2015).

FUTURE DIRECTIONS

Standard practice for transfusion SCD patients is to match for Rh (D, E, C, c, e) and K antigens. Until further cost-benefit analyses are performed, extended antigen matching for Fy, Jk, Ss, and Rh variants should be reserved for patients who are already immunized with significant alloantibodies and or autoantibodies, while considering the availability of the antigen negative unit. Equally important is the establishment of a nation-wide alloantibody registry to supplement transfusion services. Although hospital and transfusion services maintain historical alloantibody results, SCD patients commonly receive care at different sites. A complete and easily accessible alloantibody history is crucial in preventing alloimmunization.

There is considerable support for the need to engage minority communities to donate blood. Strategies to increase RBC donation among AA and other minorities should remain a top priority. They should be tailored personally to the individual need of each community with a focus on education and communication to build trust. For example, it is especially important to bring attention to female AA donors that low Hb is not a permanent disqualification and that they may be able to donate once their Hb level is higher. Theoretical frameworks can also be used to further understand blood donation practices in minority communities. Examples include the Assimilation Theory and Culture Fusion Theory, which highlights the effect of a person's cultural identity on their actions (Renzaho & Polonsky, 2013; Spratling & Lawrence, 2019).

As the cost of genotyping decreases, it would be possible to have blood group genotype of SCD patients even at birth. This information would be beneficial to identify

patients with rare blood group phenotypes, identify donors, and clarify antibody specificity. In parallel, ongoing studies can also identify susceptibility genes that contribute to SCD alloimmunization.

As treatment options for treating DHTR and hyper-hemolysis in SCD patients are limited, ongoing research should explore novel approaches in alloimmunization and DHTR. The case studies presented in this literature review represent preliminary evidence for the efficacy of rituximab, eculizumab, and tocilizumab. Further prospective studies are necessary to demonstrate their efficacy, safety, side effects, and benefit/risk ratio. Evidence-based studies and clinical trials using a larger cohort population are necessary.

It is imperative to prevent and diagnose DHTR because additional transfusions can precipitate hemolysis. However, the diagnosis and management of DHTR are not well developed. Many questions regarding DHTR remain unanswered: How should additional transfusions be administered? Is specific treatment for DHTR necessary? Studies to elucidate the mechanism of DHTR, particular triggers and markers of severity improve treatment of this condition.

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CURRICULUM VITAE

