



## Review Article

# Impact of alloimmunization on transfusion-dependent patients

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## Abstract

Red blood cell (RBC) alloimmunization can be a life-threatening complication for patients with thalassemia major and sickle cell disease (SCD) who must receive chronic therapeutic transfusions. Chronic transfusions can lead to erythrocyte alloimmunization, patients continue to develop alloantibodies due to the transference of the immunogenic antigens on the donor RBCs. Many complications are possible. Difficulty in finding compatible match units for the patients can cause transfusion delays, or present alternative risks to the patients from delayed hemolytic transfusion reactions. This review discusses the possible mechanisms, risk factors associated with alloimmunization formation and the hemolytic transfusion reactions and also describe the guideline for transfusion management of these patients, including opportunities and emerging approaches for minimizing this life-threatening complication.

## Introduction

Alloimmunization is an immune system response (alloantibody) in the body against to the donate RBC antigen. The development of alloantibodies (autoantibodies and/or anti-RBC antibodies) can significantly complicate for transfusion therapy. RBC alloimmunization can cause adverse effects, resulting in delayed or acute hemolytic transfusion reactions (HTRs) and limit the further safe transfusion. Significant morbidity and mortality risks are associated with the difficulty in cross-matching blood. Alloimmunization to RBC antigens is one of the major complications of the chronic transfusions and may require immunosuppressive drugs, splenectomy or alternative treatments [1-3]. Despite of the alloantibodies recognition as transfusion-associated risks, this review may share the knowledge about the extent causes and appropriate guideline, prevention, management and the phenomena of HTRs in chronic transfusion-dependent patients.

## Thalassemia syndrome and sickle cell disease

Thalassemia is a group of inherited anemia caused from mutations in the  $\beta$ -globin gene ( $\beta$ -thalassemia) or  $\alpha$ -globin gene ( $\alpha$ -thalassemia) that cause the defective in hemoglobin (Hb) synthesis and subsequence to cause chronic anemia in their whole life [4]. Beta-thalassemia syndromes are the group of hereditary anemia characterized by a genetic lack or deficiency in the synthesis of  $\beta$ -globin chains, including point or, more rarely or, deletion mutations to cause improper synthesis of  $\beta$ -globin, a major component of Hb, resulting in anemia [5,6]. The defect may be a complete lack of the  $\beta$ -globin protein ( $\beta^0$ -thalassemia) or markedly reduced synthesis of  $\beta$ -globin protein ( $\beta^+$ -thalassemia) [7]. Homozygous state ( $\beta$ -thalassemia major), they are both beta globin genes mutation that caused greatly impairment of  $\beta$ -globin chains production [4]. The imbalance of  $\alpha$ -globin chain production greater than  $\beta$ -globin chain, results in ineffective erythropoiesis and severe hypochromic microcytic anemia [8]. Heterozygous state or  $\beta$ -thalassemia minor or  $\beta$ -thalassemia trait, cause mild to moderate microcytic

anemia, resulting in a decrease in the  $\beta$ -globin protein synthesis by approximately 50% [4]. Intermediate state,  $\beta$ -thalassemia intermedia, occurs with mutations in both  $\beta$ -globin genes, but may be a result of: (i) one mild mutation and severe mutation in the other, (ii) two mild mutations, (iii) complex mutation with the association with  $\alpha$ -thalassemia [4]. Thus  $\beta$ -thalassemia intermedia patients can express from mild anemia to severe anemia, requiring RBC transfusions similar to  $\beta$ -thalassemia major [9]. Treatment of these  $\beta$ -thalassemia patients includes chronic transfusion, iron chelation, splenectomy and allogeneic hematopoietic stem cell transplantation. The targets of RBC transfusion therapy are to correct anemia, suppress erythropoiesis and inhibit gastrointestinal iron absorption. Previous studies have reported the alloimmunization rates of 5-30% in patients with thalassemia major, most of the RBC alloimmunization studies are from the countries in Mediterranean and Southeast Asia [10,11]. The highest rate (30%) was reported by Ameen et al. [9], in Iranian patients. In our recent study, 17.5 % of alloimmunization in these transfusion-dependent  $\beta$ -TM patients were found only in one province in Northern, Thailand [11].

Sickle cell disease is also an inherited anemia resulting from a mutation in the  $\beta$ -globin gene to cause rising of HbS [12]. When sickle Hb deoxygenates, it forms a rigid polymers within RBC that affected its shape and decrease its deformability to cause vaso-occlusion [12]. Patients with SCD have chronic anemia and increased reticulocytosis, leading to a shortened life. The vaso-occlusion can cause severe pain, end organ damage from hypoxia (especially in spleen, lung, kidney and central nervous system) [12]. This phenomenon involves many factors including hemolysis-associated reduction of bioavailability nitric oxide, oxidative stress, chronic inflammation, altered red cell adhesive properties, activated white blood cells, platelets and increased viscosity. Lifelong RBC transfusion is the major supportive treatment to ameliorate the chronic anemia and to suppress the extramedullary hematopoiesis, which would otherwise lead to severe bone disorders for these patients, and also to improve oxygen-carrying capacity, decreased viscosity of circulation and improving blood flow. RBC transfusions are the important treatment for SCD patients, 60-90% of whom receive RBC transfusions in their lifetime [13]. However, previous studies have reported alloimmunization rates of approximately 20-50% in SCD patients receiving transfusion therapy [14]. Chronic RBC transfusion regimens are confronted with serious complications [4]. These complications include iron overload, possibility to cause endocrine disorders (delayed growth, impaired sexual maturation, diabetes mellitus and parathyroid, thyroid, pituitary and adrenal insufficiency), liver fibrosis, cirrhosis, dilated cardiomyopathy [5,8] as well as alloimmunization to red cell antigens.

### Alloimmunization

The presentation of alloimmunization is similar to common infectious immunization. Both immunizations are influenced by: (i) immunogenicity of the antigen, (ii) high affinity antibody generation and (iii) inflammation in physiological environment [15]. Common events that generate general innate immune response, leading to alloantibody formation are: (i) transfusion, (ii) transplantation and (iii) pregnancy. Alloimmunization to RBC antigens is one of the important complications of regular chronic transfusions. Factors for alloimmunization generation in transfusion cases are complex and may involve in three main contributions: (i) RBC antigenic difference between the donor and recipient, (ii) the immune status of the recipient, and (iii) the immunomodulatory effect of the allogeneic RBC transfusions on the immune system of the recipient and (iv) Genetic factor [16,17].

Transfusions may introduce a large numbers of living cells and foreign antigens into the body of recipient for the variable time. An immunocompetent recipient demonstrates an immune response to the living cells and/or foreign antigens of donor, resulting in various clinical consequences and conditions. We can classify the most common antigens that involved in alloimmunization as in the following categories:

(i) Human leukocyte antigens (HLAs), HLA-class I present by platelets and leukocytes and HLA-class II present on some leukocytes; (ii) Granulocyte-specific antigens; (iii) Platelet-specific antigens (human platelet antigen; HPA); and (iv) RBC-specific antigens, as shown in tables 1-3. Hemolytic transfusion reactions may increase hemolysis risk of the newborn when this patient becomes pregnant and it can cause difficulty in hematopoietic bone marrow/stem cell transplantation and increase the risk of graft rejection in patients who becomes for these therapies in the future.

**Table 1:** International Society of Blood Transfusion: Blood group systems.

No.	Name (symbol)	Gene name(s)	No. of antigens
001	ABO	ABO	4
002	MNS	GYPA, GYPB	46
003	P1PK	A4GALT	3
004	Rh (RH)	RHD, RHCE	54
005	Lutheran (LU)	LU	20
006	Kell (KEL)	KEL	35
007	Lewis (LE)	LE (FUT3)	6
008	Duffy (FY)	FY (DARC)	5
009	Kidd (JK)	JK (SLC14A1, HUT11A)	3
010	Diego (DI)	DI (SLC4A1, AE1, EPB3)	22
011	Yt (YT)	YT (ACHE)	2
012	Xg (XG)	XG (PBDX)	2
013	Scianna (SC)	SC (ERMAP)	7
014	Dombrock (DO)	DO (ART4)	8
015	Colton (CO)	CO (AQP1)	4
016	Landsteiner-Wiener (LW)	LW (ICAM4, CD242)	3
017	Chido-Rodgers (CH/RG)	CH (C4B), RG (C4A)	9
018	H (H)	H (FUT1)	1
019	Kx (XK)	XK	1
020	Gerbich (GE)	GE (GYPC)	11
021	Cromer (CROM)	CROM (DAF)	18
022	Knops (KN)	KN (CR1)	9
023	Indian (IN)	IN (CD44)	4
024	Ok (OK)	OK (BSG, EMPRIN)	3
025	Raph (RAPH)	RAPH (CD151)	1
026	John Milton Hagen (JMH)	JMH (SEMA7A, CD108, SEMA-L)	6
027	I (I)	I (GCNT2, IGnT)	1
028	Globoside (GLOB)	GLOB (B3GALNT1)	1
029	Gill (GIL)	GIL (AQP3)	1
030	Rh-associated glycoprotein (RHAG)	RHAG	4
031	FORS (FORS)	FORS (GBGT1, A3GALNT)	1
032	JR (JR)	JR (ABCG2)	1
033	Lan (LAN)	LAN (ABCB6)	1
034 <sup>a</sup>	Vel (VEL) <sup>23-25</sup>	VEL (SMIM1)	1

[Red blood cell terminology. <http://www.isbtweb.org/workingparties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology>, St-Louis M. Molecular blood grouping of donors. *Transfus Apher Sci.* 2014; 50:175.]

**Table 2:** Main alloimmunizations in clinical practice.

	Immunization Rate	Main Alloantibody Specificity	HLA Expression
RBC	Moderate	RBC Ag RH1 and KEL1	No HLA
	Low or very low	Other RBC Ags	
Platelets	Moderate	HLA Class I /II	HLA Class I
	Low or very low	HPA	

**Table 3:** Red Cell Antigen of the greatest transfusional relevance, in addition to ABO and Rh D.

Antigen Category	Antigens
Rh antigens	C/c, E/e, VS, V HrB, HrS
Clinically significant antigens	M/N, S/s, K/k, Kp(a/b), Js(a/b), Fy(a/b), Jk(a/b), Do(a/b), Jo(a), Hy, Lu(a/b), Di(a/b), Co(a/b)
Rare antigens	Lu(8/14), In(a/b), Cr(a-), Sc(1/2), Tc(a/b), Yt(a/b)
Variants	DIIIa/DIV, E/e variants, Fy(a-b-), Fy(x), JknullFinn, U, UVAR, MNS, Mia

## Alloimmunization to RBC antigens

ABO blood groups are the most important factor for the major compatibility in safety blood transfusion. The International Society of Blood Transfusion (ISBT), in A and B blood group antigens are now recognizes over 300 blood group antigens that can be identified using serology and most of which acknowledges 33 genetically discrete blood group systems [18]. Small integral membrane protein 1 (SMIM1) is a new blood group gene, it was proven responsible for the Vel antigen expression [19-21]. Antibodies against to these over 300 antigens have caused significant clinical consequences, among which they can accelerate RBC destruction along with the corresponding antigen destruction.

Platelet and neutrophil antigens are less well known than the RBC, but they also play an important role in the transfusion. There are many different antigenic systems on the surface of platelets (PLTs). These polymorphic structures located on the membrane of PLTs are called HPAs. They are important for clinical medicine [22] and now 33 PLT antigens have been identified [23]. Twelve of HPAs are grouped in the biallelic systems: HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, and HPA-15 [24]. HPAs, single-amino-acid polymorphisms are immunogenic and able to stimulate the immune response after transfusion and in pregnancy [25]. After transfusion, HPA alloantibodies can cause post-transfusion purpura, PLT transfusion refractoriness, passive alloimmune thrombocytopenia or transplantation-associated alloimmune thrombocytopenia [22,26]. Antigens of the HLA system are expressed on white blood cells (WBCs), as the same as PLTs. Alloimmunization in this system can lead to PLT refractoriness, lower survival rate and impaired the function of transfused PLTs [27]. The majority (80%-90%) of HLA antibodies is directed against with HLA class 1 antigens [27]. This HLA class 1 antigens were named A and B antigens [27]. Immune response of HLA is stimulated by contact with foreign antigens, such as transfusion of blood components containing WBCs, fetomaternal blood transfusion during pregnancy and transplantation [27]. Patients receive RBC transfusions that are WBC-reduced by filtration or buffy coat depletion. Both methods can reduce or prevent alloimmunization against HLA but the filtration method is more efficient to reduce WBCs [28].

The human neutrophil antigens (HNAs) system is the polymorphic structures located on the membrane of neutrophils and also important in transfusion medicine [29]. Five HNA-systems (HNA-1-HNA-5) have been identified [30,31]. In general, HNA antibodies can stimulate transfusion reactions and autoimmune neutropenia [32]. The autoimmune neutropenia, wherein the autoantibodies in the recipients' blood are being activated against their own neutrophils, mostly occurs in infancy [32]. Transfusion-related acute lung injury (TRALI) is one of the most severe events in transfusion reactions. This is caused by HLA antibodies or HNA antibodies in the plasma of the different blood components, but also caused by transfused biologically active lipids or transfused leukoagglutinin or other soluble factors [33]. Most often, fresh frozen plasma or PLT concentrates are demonstrated in TRALI cases. Transfusion reaction results from the complexity of the immune response against antigens present on the transfused RBCs but that are lacking on the recipient's. The reaction can be mild, moderate or strong. It can occur immediately or with some delay, and in rare cases the reaction may be fatal. The antibody productions in these processes are also problematic in the long term, causing transfusion incompatibility for the patients and materno-fetal incompatibility in pregnancies [34].

There are many practical ways to reduce HLA alloimmunization. The most important practice is the inactivation or reduction of WBCs contaminating cellular blood components by using the filtration or ultraviolet B irradiation [28,35]. The length of storage of the RBC component before transfusion is important. The fresh donated RBCs can induce HLA alloimmunization while RBCs stored at least 15 days seem to

induce immune tolerance against foreign HLA antigens [28,36]. Mincheff reported RBCs storage at 15 days leads to full disintegration of granulocytes [36]. The protein-free media storage for 5-7 days results in the impairment of donor T cells function [36].

Alloimmunization causes from the contact with non-autogenous antigens, it can happen and depends on various factors [37-39]. Thus, antigen systems on RBCs are very important in this reaction. Most RBC antigens are protein antigens including with the carbohydrate ABO-system and the natural occurring immunoglobulin M antibodies (ABO-isoagglutinins) [25]. The most immunogenic RBC protein antigen is Rhesus-D, which can lead the immune response up to 70% in the Rhesus-D-negative individuals. It can cause hemolysis and fetal death in pregnancy case [25]. D is the most immunogenic RBC protein antigen and clinically important in RBC transfusions after the ABO antigens. Other Rhesus antigens, included Kidd, Duffy, MNS, and Kell antigens, may also be the clinically importance, even though their immunogenicity is lesser than of the Rhesus-D (from 0.03%-10%) [40,41]. All alloantibodies can react with these antigens to cause hemolysis following the second contacted with these foreign antigens in the recipient's blood. All of these alloantibodies can be detected by pre-transfusion screening of the recipient's blood by using a RBC panel with at least three cells. To specify a certain antibody, more panel cells are used. In normal conditions, the compatibility testing is performed by testing the recipient's blood against a screening cell panel (antibody screening), as well as against the red cells of blood components for transfusion (cross-match testing) for all patients. When any antibodies have been identified, donor blood with the corresponding antigen should be selected for transfusion. The early detection of alloantibodies depends on the necessity of early re-investigation for repeated RBC transfusions to patients. Otherwise, some alloantibodies can drop out of the detection limit after a longer series of transfusions.

A research study demonstrated alloimmunization risk did not depend on patients' gender while another demonstrated higher incidence in females [42,43]. Verduin et al. [44], demonstrated that female patients with sickle cell disease develop alloantibodies more frequently than men, while in thalassemia cases both genders have the same risk. When more transfusions were made, the higher alloantibodies risk existed [43]. This is the major problem of patients with received RBCs as chronic transfusion therapy. There are some specific antibodies in the certain populations, as different antigens have been observed within populations [45]. For example, Africans often developed more alloantibodies against the MNS and Duffy, while Polynesians developed mostly alloantibodies against the Kidd [46]. When RBC alloantibodies were formed in the recipients, the higher risk for developing hemolytic transfusion reactions occurred. The rate of the alloimmunization can be reduced when providing the extended phenotyped RBCs (0-7%) [47], and the earlier age (<3 yr) initiation of RBC transfusion [48]. Splenectomy has been demonstrated as a risk factors for alloimmunization formation [11,17]. Singer et al reasoned that removed spleen led to non-filtering of antigens responsible for alloimmunization.

The condition of the donors can affect alloimmunization. Two important considerations are inflammation and donor immunogenicity status. (I) Inflammation: From research evident, immunogenicity of antigens can be enhanced by inflammation [49]. In blood products, inflammatory factors can occur in two ways: (i) Preparation of blood products (especially in leukoreduction) might influence proinflammatory molecule during storage (ii) From dangerous donors, which might demonstrate high proinflammatory factors accumulated in blood products. Inflammatory response can be stimulated by several endogenous factors, including leucocytes, altered or ageing cells (RBCs, platelets) and their residues, chemokines, and cytokines. All of these molecules are now demonstrated as biological response modifiers (BRMs). These BRMs can be infused with plasma or secreted in stored platelets [50]. They may also influence as antigens presenting cells of the recipient. The role of iron from the RBC destruction,

in creating the iron-mediated inflammation creating has been proven [51]. It has been suggested that red blood cell component (RBCCs) collection and preparation could increase the proinflammatory state of the RBCC. In this way, platelets, RBC, leukocytes, their residual and plasmatic molecules can generate some BRMs. The main contributors are leukocytes and their residual, especially if no leukoreduction has been done. In this way, leukocytes and platelets seem to have synergistic action in anti-HLA immunization [52]. Immunization without leukoreduction transfusion can occur against HLA-Class I (by platelets and leukocytes) and HLA-Class II (not expressed by platelets). (II) *Residual leukocytes and other components*: The blood product of the transfusion to a recipient is composed with 3 major *components*: (i) RBCs and platelets: main product(s); (ii) Anticoagulant, additive solutions and plasma residual; (iii) leukocyte, leukocyte debris and residual leukocytes: all carry HLA and non-HLA antigens. Some residual allogeneic leukocytes (potentially inflammatory factors secreting cells) can induce alloimmunization against RBC or platelet antigens [53,54]. However, intensive leukoreduction transfusion has been demonstrated tolerance or even absence of antibody formation or alloimmunization [53,55]. A study of reduce alloimmunization of platelets produced data on HLA Class I platelet immunogenicity showing that leukoreduction and/or leukocyte inactivation significantly reduced alloimmunization [53].

There are also many factors of concern regarding recipients in alloimmunization of RBC transfusion. (i) Environmental factors: Inflammatory state can influence alloantibodies. There are many animal experimental evidences that the recipient status favors the occurrence of alloimmunization [56-58] and that is also possible in humans [59]. Many research studies have clearly shown inflammation enhanced immunization occurring [49,56,57,60] and some certain transfusion conditions induce inflammation. (ii) Genetic risk factors: Tatari-Calderone et al. [61], demonstrated the marker of alloimmunization in the gene encoding for Ro52 protein, known as Sjögren syndrome antigen 1 (SSA1) and tripartite motif (TRIM21) and found the opposite association with lupus; the same variant of a polymorphism (rs660) was associated with lupus incidence and also associated with induction of tolerance to RBC antigens during early childhood. They propose lupus/ autoimmunity susceptibility loci may play roles of additional molecules in various aspects of alloimmunization induced by transfusion and pregnancy [61] (iii) Recipients' tolerances to alloantigens: Some patients do not develop any alloantibodies even after repeated RBC transfusions, despite high frequencies of antigen mismatch [13,62]. In mouse models of RBC immunization, CD25+ T cells were demonstrated as the active mediator tolerance to transfusion [63,64]. In sickle cell disease patients, the Treg and Breg compartments are important. Alloimmunized patients demonstrated low Treg cells, reduced IL-10 levels and enhanced the IFN- $\alpha$  production compared with tolerant patients [65,66]. Absence of Treg cells favored alloimmunization against platelet donor antigens and thrombocytopenia in transfused recipients [67]. Alloantigens can induce both immunization with T helpers and Tregs cells, leading to alloimmunization or specific tolerance induction, respectively. Non-HLA genetic risk factors were supported this hypothesis and associated with lacking immunity to caused tolerance ways and apoptosis. Overall, alloimmunization can be demonstrated as a breach of tolerance and alloimmunity against RBC have been linked together in some studies [62,68].

Alloimmunization prevention in transfusion-dependent patients may include cytokine blockade or the processing of immune cell-depleting agents [14]. In general the different precautions to avoid alloimmunization are: (i) Blood group of patients was determined precise as possible, especially in preexisting alloantibodies recipients. (ii) Screening for alloantibodies should be performed before every transfusion. (iii) Recipients must not receive bar RBCs antigen when such patients had formed alloantibodies and weak alloantibody. (iv) The patient's transfusion history must be examined, it is extremely important especially in polytransfused patients.

## Hemolytic Transfusion Reactions (HTRs) [69]

Intravascular HTRs are characterized by the antibody-mediated hemolysis within the circulation and releasing hemoglobin into the circulation. Intravascular HTR is the rapid hemolysis and will destroy most RBC within 10 minutes. This rapid hemolysis is caused by immunoglobulin M (IgM) antibody that can activate the complete classical complement pathway associated with complex membrane formation and puncturing of the RBC membrane [69]. The common signs are chills, shock, hypotension, hemoglobinemia, hemoglobinuria and addition with complications of disseminated intravascular occlusion and renal failure. The most common antibodies that cause intravascular HTRs are the anti-A, -B, -AB of the ABO group system, while antibodies of anti-PP1Pk, -Vel, Lewis and Kidd have been demonstrated on rare occasions [69].

Extravascular HTRs are the most HTRs that do not cause from the ABO group system. Immunoglobulin G (IgG) antibody is involved in the extravascular HTRs [69]. It does not bind with complement or bind insufficient C3 to initiate the hemolytic pathway. It may be immediate (occurred within a few hours of transfusion) or delayed (occurred within a few days of transfusion) [69]. The main signs are similar to intravascular reactions but less severe. Hemoglobinemia and hemoglobinuria may present and hyperbilirubinemia is a common characteristic. Extravascular HTRs may be caused by IgG antibodies (IgG3 and IgG1) from the Rh system, those do not activate complement. RBCs coated with IgG were attached to the Fc receptors on macrophages were phagocytosed in the spleen. IgG antibodies such as Kidd and Duffy systems, may cause C3 binding to the transfused RBCs, but are insufficient to cause intravascular hemolysis. RBCs coated with IgG and C3b will be sequestered by macrophages in the liver and also in splenic macrophages [69]. Intravascular HTRs and extravascular HTRs may be occurred concurrently in some cases. Immediate or acute extravascular HTRs occur either during the transfusion or within a few hours [69]. The serologically detectable antibodies in the plasma of the recipient before the transfusion inevitably causes delayed reactions or immediate extravascular HTRs, those are usually avoidable.

### Delayed HTRs

Delayed HTRs occurs in patients who have been immunized to unusual antigens but while the antibody levels have dropped too low to be detected the serological reaction, but nevertheless can cause intra- or extravascular hemolysis. Typically this delayed HTR (DHTR) occurs approximately 5 to 7 days after the transfusion, while in the extreme cases may be in 3 days and late as 14-23 days [69]. Clinical symptoms often involve fever, jaundice, low hemoglobin level and hemoglobinuria while renal failure is rare [69]. These delayed reactions can only be detectable by the serological tests, especially by a positive direct antiglobulin test and may be defined as delayed serological transfusion reactions, and cause no significant morbidity. The antibodies most frequently involved in delayed HTRs are Rh, Kidd, Duffy, Kell, MNSs systems, though other blood group antibodies are occasionally implicated [69]. DHTR development is the most serious of alloimmunization in these transfusion-dependent patients, which can be life-threatening.

### Detection and identification of antibodies

Transfusion-dependent patients are those who require frequent and long-term transfusion support to sustain their life, including patients with severe thalassemia syndromes, SCD, severe aplastic anemia, myelodysplastic syndromes and other congenital or acquired chronic anemia. These conditions often cause alloimmunization in transfusion-dependent patients more than other patient groups, with the highest rates being in SCD.

Alloantibody detection and identification are the fundamental in transfusion practice to provide information which aids in the matching compatible for blood

transfusion. Red cell antibodies are detected by using serological tests. The certain characteristics can be indicated their clinical significance as: antibody strength, mode of reactivity indirect antiglobulin test, thermal range, specificity, immunoglobulin class, IgG subclass, affinity and ability to bind complement. Thermal range is important if this antibody does not react at 37°C, it is not significant enough to cause in vivo red cell destruction and will have no clinical effects. Other factors are the quantity and distribution of antibody to the target antigen on red cell membrane, the quantity of IgG and/or complement bound to the RBC and the presence of target antigen in tissues and/or body fluids. Although it is often easy to predict and identify these alloantibodies by serological tests, these tests do not distinguish between the clinically significant and the clinically benign alloantibodies. In many case conditions, alloantibody identification can be a difficult and time-consuming process delaying the patient care.

There are guidelines for ABO, D typing and alloantibodies detection [70], while there is very little guidance for the procedures necessary for alloantibodies identification. When the positive antibody screen is detected, there are many procedures that can be followed. Thus, each laboratory should set a policy outlining its procedures. There are more sophisticated techniques those may be necessary for some antibodies. It is important that routine techniques are not oversensitive. The most problems in the identification process result from a complex mixture of antibodies within routine systems, such as Rh, Kell, Kidd, Duffy, MNS, Le, Lu, P, or the antibody to a high-incidence antigen with or without underlying antibodies. In both of these cases, all, or at least the majority of RBCs matched against the serum are positive and difficult to find the compatible blood. The racial background of a patient is useful to know because some rare phenotypes are found exclusively in certain populations, including In(b-) in Asians and S-s-U-, Js(b-), hrS- in blacks. While k, Kpb, Yta, Vel, Coa, and Lub are the high incidence antigens that Caucasian patients are more likely to have antibodies. The clinical history and previous serological findings of the patients are the most useful information. Knowledge of the expected phases of reactivity for different antibodies will be a guide to certain specificities. Strength of reactivity may be a clue for the type of present antibody while a very strongly reacting antibody is more effect clinically significant. If they present weak reactivity in the negative cells may not react with the antigen but might cause from the weaker expression of Ch, Rg, Cs<sup>a</sup>, Kn<sup>a</sup>/McC<sup>a</sup> (complement receptor 1 [CR1]-related). Some antibodies react more weakly with cells carrying a single dose of antigen such as M, N, S, s, Jka, and Jkb. Partial or total hemolysis of test red cells may be occurred with some antibodies, including Le, Jk, PP1Pk, Vel, H (made in Oh), must be marked as a positive reaction. These factors should be considered as useful clues and careful observation of them, these can aid in the antibodies identification process.

### **Transfusion management strategies to prevent alloimmunization and DHTR**

The main immunological complication of the received chronic transfusion in both  $\beta$ -thalassemia major and SCD patients is alloimmunization against RBC antigens, making it difficult to match compatible transfusion and limiting further therapies, leading to cause post-transfusion hemolysis being a life-threatening concern in transfusion medicine. Alloimmunization in SCD patients is more frequent occurrence than other conditions [71].

In  $\beta$ -thalassemia, the decision to start transfusion therapy should be based on the presence of hemoglobin (Hb) levels <7 g/dL measured on two occasions at an interval of more than two weeks. All of these contribution factors including folic acid deficiency, coinheritance of glucose-6-phosphate dehydrogenase (G6PD) deficiency, infections and blood loss must be discarded.

However, in case with Hb level (>7 g/dL) should be considered with the following clinical criteria: poor growth, facial changes, spontaneous fractures and clinically



significant extra-medullary hematopoiesis. The RBC transfusions requirement can start as early as six months old [7]. The decision to start regular RBC transfusions should not be delayed in the first year of life, after that the risk increases for alloantibodies development and difficulty in finding compatible matches [9,48,72]. The recommended treatment of RBC transfusions is to maintain the pre-transfusion level of hemoglobin between 9 and 10.5 g/dL [7,72]. This transfusion regimen can promote normal physical activities, proper growth, adequately of bone marrow activity inhibition and minimizes iron accumulation [73,74]. In cases with hemoglobin level between 11 and 12 g/dL at pre-transfusion, there is a risk for heart disease, extra-medullary hematopoiesis and splenomegaly. Some patients complain of lower back pain near the time of RBC transfusion, which may indicate a need to maintain higher pre-transfusion Hb levels. Extra-medullary hematopoiesis occurs when adequate suppression of bone marrow activity cannot be achieved [72]. The mean target Hb should be at 12 g/dL and in post-transfusion Hb should be maintained at a maximum between 14 to 15 g/dL, because the higher of the post-transfusion Hb level is the greater the hyper-viscosity, which may increase risk of stroke [72]. All patients who start to receive regular RBC transfusion should be vaccinated against hepatitis A and B and should be assessed their cytomegalovirus [6]. Blood type of patients must be determined with RBC phenotyping for at least the C, c, D, E, e, and Kell antigens useful for identification and characterization antibodies in the case of subsequent immunization and the extended phenotyping should be performed including the antigens of other blood systems such as Duffy and Kidd [72]. Baseline phenotyping and genotyping of patients is important, and checked against development of new antibodies to avoid administering the packed RBC transfusion with the corresponding antigens [72]. The amount of RBCs used in transfusion depends on various factors including patient weight, target of Hb levels and hematocrit of the blood pack. For example, the study of Rachmilewitz and Giardina demonstrated that clinically stable patients can be infused with approximately 8-15 mL/kg body weight of RBCs over a period of 1-2 hours [6]. Using leukocyte depleted/filtered RBCs is recommended for all patients to reduce alloimmunization, febrile non-hemolytic transfusion reactions and cytomegalovirus infection [6]. The leukocyte depleted/filtered RBC is generally indicated for patients with repeated transfusion reactions and IgA-deficient patients [72]. Non-splenectomized patients are generally need for transfusion higher (approximately 30%) than in splenectomized patients [75].

The most serious of alloimmunization in these patients is the risk of DHTR development, which can be life-threatening. In many DHTR cases of SCD patients, hemoglobin level drops lower than pre-transfusion level which caused by hemolysis of the transfused RBCs associated with patient's own RBCs hemolysis; this condition known as hyperhemolysis. Continuous RBC transfusions may increase RBC hemolysis and further severe anemia. This hemolysis of own RBCs in DHTR of SCD patients is caused by the presence of autoantibodies [76]. However, many cases of DHTR/hyperhemolysis condition have been reported in the absence of alloantibodies or autoantibodies detection. We know that alloimmunization is the trigger in autoantibody production. Alloimmunization of erythrocytes is involved in many processes as multiple step reactions, including RBC antigen recognition, processing and presentation of antigen by HLA class II to TCR, CD4 helper T cells activation, interaction of T and B cells and finally B-cell differentiation to produce antibody into plasma cells.

## Conclusion

Now, molecular tools to type the most blood group variants have been developed [77], but challenges still remain for the diagnosis, prevention and management of alloimmunization in transfusion-dependent patients. Understanding the mechanisms and associated risk factors of alloimmunization will aid in development of strategies to prevent and inhibit production of antibodies and try to minimize its life-threatening

complications in transfused patients. With regard to current transfusion management in transfusion-dependent patients, we recommended performing an extended phenotype for all transfusion-dependent patients at diagnosis, careful monitoring of laboratory tests before and after every transfusion, and concomitant use of an electronic data system for patient transfusion history. Research studies should identify the role of genetic modifiers of alloimmunization and identify the susceptibility genes that contribute to alloimmunization formation.

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