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Review

Red cell transfusion in paediatric patients with thalassaemia and sickle cell disease: Current status, challenges and perspectives

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ABSTRACT

Notwithstanding the high safety level of the currently available blood for transfusion and the decreasing frequency of transfusion-related complications, administration of labile blood products to paediatric patients still poses unique challenges and considerations. The incidence of thalassaemia and sickle cell disease in the paediatric population may be high enough under specific racial and geographical contexts. Red cell transfusion is the cornerstone of β-thalassaemia treatment and one of the most effective ways to prevent or correct specific acute and chronic complications of sickle cell disease. However, this life-saving strategy comes with its own complications, such as additional iron overload, alloimmunization and haemolytic reactions, among others. In paediatrics, the dependency of the transfusion outcome upon disease and other recipient characteristics is more prominent compared with the adults, owing to differences in developmental maturity and physiology that render them more susceptible to common risks, exacerbate the host response to transfused cells, and modify the type or the clinical severity of the transfusion-related morbidity. The adverse branch of red cell transfusion is likely the overall effect of several factors acting synergistically to shape the clinical phenotype of this therapy, including inherent donor/blood unit variables, like antigenicity, red cell deformability and extracellular vesicles, as well as recipient variables, such as history of alloimmunization and inflammation level at time of transfusion. This review focuses on paediatric patients with β-thalassaemia and sickle cell disease as a recipient group with distinct transfusion-related characteristics, and introduces new concepts for consideration, not adequately studied and elucidated so far.

1. Introduction

The most common monogenic diseases on a global scale include hereditary disorders of haemoglobin (Hb), mainly thalassaemia and sickle cell disease (SCD). The sickle mutation is allocated on the *HBB* gene (Glu6Val, βS) and results in the intracellular polymerization of the deoxygenated Hb molecule, the pathophysiological hallmark of all clinical forms of SCD. Pathological Hb polymers potentially damage the red blood cell (RBC) membrane forming abnormal rigid sickle-shaped cells. These malformed cells can cause vaso-occlusion (VOC) leading to distal tissue ischaemia and inflammation [1] that underlie acute painful sickle-cell crisis. The chronic haemolysis with acute exacerbations, and the progressive vasculopathy, oxidative stress and organ dysfunction, which start in infancy and continue throughout life, result in increased morbidity and premature death [2]. The commonest and most severe form of SCD is the homozygous HbSS which is also referred as sickle cell anaemia (SCA). Other forms of SCD include compound heterozygous conditions, such as cases of HbS mutation co-inherited either with β-thalassaemia mutations (HbS/β°-thalassaemia or HbS/β^{+}-thalassaemia), or HbC mutation (HbSC) and HbS with other beta-globin variants such as HbSD or HbSOArab [3].

The clinical forms of thalassaemia disorders are caused by mutations affecting the α and/or β peptide chains of Hb. The clinical presentation depends on the resulted imbalance in the α/β-globin chain ratio and the subsequent chronic haemolytic anaemia, ineffective erythropoiesis, compensatory increased gastrointestinal iron absorption

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and medullary expansion outside the bone marrow. During the last decade, a trend for classification of the various thalassaemia syndromes has been established that is largely based on clinical and management criteria, rather than on molecular characteristics. Currently available treatment for both thalassaemia and SCD includes blood transfusions applied either regularly or sporadically according to the clinical presentation of the diseases, starting from infancy (28 days-1 year) or childhood (< 18 years). Thalassaemia patients are nowadays categorized as having transfusion-dependent (TDT) or non-transfusion-dependent thalassaemia (NTDT) [4]. TDT patients are not able to synthesize enough Hb to survive, unless regular RBC transfusions are given. NTDT patients also need transfusions, but only sporadically or at regular intervals for a certain period of time. TDT mainly includes patients with β-thalassaemia major (βTM) and severe forms of HbE/β-thalassaemia, whereas NTDT includes β-thalassaemia intermedia, HbH disease and some types of HbE/β-thalassaemia [4]. It should be noted that the clinical criteria used for the classification of thalassaemia patients can change over time based on both progression of the disease and advances in its clinical management. Consequently, the TDT/NTDT classification does not reflect a lifelong classification, but only the current clinical status of a patient that may change [4,5].

Paediatric patients have the longest potential lifespan which makes appropriate transfusion policy and safety of paramount importance in this population. Newborns (< 28 days of life), infants and young children are small sized, immature and particularly vulnerable in specific transfusion complications. Transfusing infants and children, therefore, does not only imply smaller transfusion volumes. There are unique challenges, considerations and host responses to transfused blood, resulting, among other, by a range of physiological differences compared with the adults, including higher average Hb concentration and oxygen requirements. Moreover, children at different ages also vary not only in blood volume, but also in metabolic rate and body surface area to mass ratio. These disparities lead to specific transfusion indications and doses. Specialized components and additional safety measures are applied for transfusion to different paediatric patient groups and for different clinical indications, including thalassaemia and SCD.

2. RBC transfusion for the treatment of Thalassaemia in paediatrics

Transfusion therapy is the cornerstone of the optimal management of thalassaemia. By providing normal allogenic RBCs, ineffective erythropoiesis is suppressed along with most of the subsequent catastrophic pathophysiological mechanisms of the disease [6]. However, the chronic transfusions introduce secondary complications, mainly related to alloimmunization and iron overload/toxicity (see below), which contribute to high morbidity rate [4]. Nowadays, implemented improvements in transfusion practices and iron–chelation therapies have decreased the incidence and severity of co-morbidities, increasing substantially the life expectancy of thalassaemia patients [7–9].

The initiation of transfusion therapy in a child with thalassaemia remains a delicate and critical decision made by haematologists and other specialists, after the overall clinical evaluation of the patient. Despite the fact that the genotype can provide some initial indication about the expected clinical severity of the disease and the likelihood of developing TDT, the decision is made at clinical level. As the borderline between thalassaemia intermedia and major is sometimes blurred, it is not always possible to predict the evolution of the disease to TDT or NTDT. Hb level by itself is not enough to drive the critical decision to start a regular transfusion program, and thus, additional criteria are taken into consideration, including whether the child is thriving or not, the height velocity, weight gain and spleen size. Mistreated children over-transfused to the point of iron toxicity, which would probably have been better without regular transfusion at all, may hopefully belong to the past, but should guide us towards future decision making.

In clear cases of thalassaemia major, the presence of persisting anaemia (with Hb < 7 g/dL at least on two occasions in a 2-week interval), along with clinical manifestations that include fatigue, poor feeding, developmental delay or regression, growth deceleration, and symptoms or signs of cardiac dysfunction determine the initiation of regular transfusion therapy. Coexistence of anaemia-contributing factors, such as iron deficiency, infection or glucose-6-phosphate dehydrogenase (G6PD) deficiency, are also taken into consideration. In TDT patients, regular RBC transfusions start from infancy or early childhood, usually before the age of three. Despite evidence that late initiation of regular transfusions in those cases increases the risk of RBC alloimmunization, providing low risk RBC units in alloimmunized patients remains a challenge, especially in the developing countries [9,10].

In children with NTDT (often intermedia), however, transfusions are usually given in adulthood to manage or prevent some of the complications of the disease and only sporadically during childhood, in order to treat acute anaemia resulted by a transient factor, such as viral infection or surgical intervention [9]. Then, the child who can maintain (at steady state) Hb at satisfactory concentration is reevaluated. In cases of fall in height velocity or peak bone mass, regular transfusions are given until *epiphyseal plate closure*. Re-evaluation of the possibility to gradually withdrawn regular transfusions is then carried out, given that many patients can adapt to chronic anaemia without substantial bone marrow stress [11]. Nevertheless, it is worth mentioning that according to several observational studies, regularly transfused NTDT children, especially with β-thalassaemia intermedia, have better growth variables, while the development of morbidities (such as hypercoagulability, silent strokes, pulmonary hypertension and extramedullary hematopoietic masses) in regularly transfused NTDT adults seems to be lower [4,5,11–14]

In chronic transfused patients, maintaining Hb levels above 9–10.5 g/dL is sufficient to both inhibit bone marrow expansion and minimize transfusion-related iron overload [6,15]. In patients with cardiovascular disease and/or excessive extramedullary haematopoiesis, transfusion thresholds of 11–12 g/dL have been reported to be beneficial. Regarding transfusion intervals, they usually range from 2 to 4 weeks according to the severity of anaemia and the clinical response to therapy, along with personal decisions of the patient and socioeconomic conditions, including access to hospital and blood supply. The volume of RBCs transfused into children is calculated by using a formula that incorporates the patients' weight, the desirable and the actual Hb levels [16].

3. RBC transfusion for the treatment of Sickle Cell Disease in paediatrics

Blood transfusions, either simple, exchange, or chronic, in SCD remain one of the most effective treatments for both acute and chronic complications. The transfusion of allogenic RBCs in these patients corrects anaemia and, at the same time, dilutes out the number of sickle cells that underlie VOC and vascular damage [17]. The most common goal for HbS concentration is less than 30%, a threshold that is based mainly on expert consensus rather than randomized trials [18]. It is worth mentioning that although children are more able to tolerate large increases in Hb than adults [17], the most effective way to achieve this HbS threshold is by exchange transfusion, which removes sickle cells without the risk of hyperviscosity [19]. Thus, simple RBC transfusions as well as exchange transfusions are given acutely in severe clinical events to offer immediate improvements in blood flow and tissue oxygenation, among other. Chronic transfusions are usually given monthly to prevent several long-term complications, such as strokes, in both children and adults. Apart from replacing the non-deformable sickle RBCs with normal ones, the chronic transfusions further suppress their long-term formation [3]. According to the Cooperative Study of Sickle Cell Disease's infant cohort, 35% of the enrolled children with HbSS disease received at least one blood transfusion by the age of five [20]. Moreover, it has been estimated that nearly half of the paediatric SCD

patients will receive a blood transfusion during their care [21,22], while the rate of transfusions among hospitalized children is highest for those with neutropenia, agranulocytosis and sickle cell crisis [23].

Clinical indications for simple transfusions include severe symptomatic acute anaemia of any cause, with substantial (> 2 g/dL) decrease in Hb levels below patients' baseline. Acute anaemia has been identified as the most common reason for transfusion in a large infant cohort, including splenic sequestration in 34.6% of the cases, followed by acute chest syndrome (ACS) in 27.5% and infection in 9.8% of them [24]. Additional causes of acute anaemia include RBC aplasia caused by human parvovirus B19, hepatic sequestration, hyperhaemolysis [19], severe VOC, and infections such as malaria and influenza [3]. It is worth mentioning that end-organ damage has been mostly associated with acute exacerbations of chronic anaemia. The risk of silent cerebral infractions increases with increasing frequency of acute illnesses, during which the Hb levels fall to less than 6 g/dL. The incidence of this complication has been recently found higher than previously reported [25].

No benefit of transfusion (in respect to the length of hospital stay and other parameters) has been demonstrated in SCD with VOC pain crisis [26]. However, according to the MAGiC trial approximately 20% of the children admitted to the hospital for a pain crisis received a transfusion. Hb concentration of less than 6.3 g/dL was found strongly associated with transfusion [20]. Although a recent pilot study suggested a potential benefit from early transfusion at onset of VOC [26], transfusion in SCD patients with acute pain is limited to those with coexisting severe acute anaemia [17].

In children and adults presenting with ACS, both simple and exchange transfusions are frequently prescribed, as there has been an association with better outcome [27,28]. In the MAGiC randomized controlled trial, 73% of children that developed ACS received transfusions during hospitalization for VOC pain crisis [20,29]. However, no randomized trial has compared simple and exchange transfusion in these cases. According to the guidelines of the British Society for Haematology, a simple transfusion is recommended in ACS cases with a) Hb level > 1 g/dL below baseline, b) oxygen saturations lower than 93% or increasing oxygen requirements, while an exchange transfusion is recommended a) in cases that get worse despite the initial simple transfusion and b) in cases of worsening hypoxia, increasing tachypnea, thrombocytopenia or worsening anaemia, multilobe involvement on chest X-ray, neurological complications and cases in need of mechanical ventilation [17]. Nevertheless, in the MAGiC trial that examined the RBC transfusion patterns in children hospitalized with VOC [29], no significant association with the two criteria for prescribing RBC transfusions in ACS, namely, oxygen saturations < 90% despite supplemental oxygen and decrease in Hb of 1 g/dL or more was found.

Regarding acute neurological events, it is well known that patients developing acute ischemic stroke are treated with exchange transfusion to rapidly decrease HbS levels below 30%, to improve the oxygen supply and to lower the risk of recurrence [30]. Initial simple transfusion, especially in coexisting severe anaemia (Hb < 6 g/dL) is an alternative [17]. A similar approach is applied in haemorrhagic stroke, although occasionally, urgent neurosurgical intervention takes priority over transfusion. In general, any paediatric patient presenting with new, unexplained focal neurological signs should be considered for urgent transfusion, potentially with a target HbS < 30% [17].

Concerning perioperative management, patients with SCD benefit from preoperative transfusions that increase Hb to 10 g/dL. It is worth mentioning that the multicenter Transfusion Alternatives Preoperatively in Sickle Cell Disease (TAPS) trial, which randomly assigned patients to preoperative transfusion versus standard care, was prematurely discontinued, as patients receiving standard care (no transfusions) developed severe clinical complications, especially ACS [31]. Exchange transfusion, with a target HbS < 30%, is indicated for patients undergoing high risk surgery (e.g. major neurosurgery, cardiothoracic) and for those at high risk of perioperative complications (e.g. patients with severe organ damage or history of complications) [17].

Indications for chronic transfusion mainly include secondary and primary stroke prevention in both children and adults with SCD. The rate of stroke recurrence is high in both populations [32]. Most importantly, discontinuation of regular prophylactic transfusions is associated with stroke recurrence and increased mortality [33]. Regarding primary stroke prevention, it is well established that chronic transfusion reduces the risk of stroke in children with raised trans-cranial Doppler (TCD) velocities [34]. The reduction was estimated to be around 90% for stroke risk in children with TCD velocities > 200 cm/s, when HbS was maintained < 30% [34,35]. However, it is not yet clear for how long transfusion should be continued in these children. The STOP 2 trial showed that withdraw of regular transfusions after 30 months results in a recurrence of stroke risk [36], suggesting that transfusions are required indefinitely. It is worth noticing that hydroxycarbamide has been found as effective as transfusion for primary stroke prevention in children with abnormal TCDs, provided that transfusions are given for at least 1 year and no severe intracranial vasculopathy is present [17]. The optimal management of children with silent (subclinical) cerebral infarction but normal TDC velocities is not clear yet, as according to the Silent Cerebral Infarct Transfusion (SIT) trial, the benefit of starting chronic transfusions in these children seems to be modest [37]. Thus, regular blood transfusions are offered in practice to all children with SCD and a history of stroke, typically of indefinite duration. However, it should be taken under consideration that typical transfusion complications (see below) along with impaired venous access or parents' personal choice can render regular transfusions not applicable [17].

Apart from preventing neurological complications, chronic transfusions are also given to children with a history of splenic sequestration to prevent recurrence, typically after two episodes of acute splenic sequestration requiring transfusion. In these cases, early splenectomy is indicated or alternatively, regular monthly transfusions are given until a splenectomy can be safely performed, or until the tendency to sequestration is diminished [17].

4. Adverse effects of RBC transfusion in paediatrics

Transfusion of blood and labile blood products in children is still associated with risks related to both developmental immaturity and transfusion biology *per se*. Secondary to differences in size and physiological systems, a child may incur variable risks or susceptibility to common risks compared with adults, which deserve careful consideration [38]. The transfusion-associated hyperkalaemia for instance (that may follow a therapy by whole blood, irradiated or older RBC units), poses a significant risk of cardiac arrest in children and infants receiving exchange transfusion due to both volume/size and the developing renal function [39]. The adverse effects of transfusion fall into several broad categories, including those of iron overload, allosensitization and haemolytic reactions that are an important source of transfusion-related morbidity despite the well-defined procedures to prevent them [40].

4.1. Iron overload and toxicity

Under certain conditions of primary (disease-induced) or secondary (treatment induced) iron overload, the excess of iron overwhelms the binding capacity of transferrin and hereafter, non-transferrin bound iron (NTBI) is produced [41]. By promoting oxidative stress, expression of adhesion molecules [42], inflammation and infection (development of ferrophilic pathogens), NTBI may be toxic at cellular and systemic level. The increased rate of haemolysis in βTM and SCD leads to steadily elevated basal levels of NTBI [43]. In addition, there is an inappropriately low expression of hepcidin (the master regulator of iron homeostasis [44]) in βTM, which further exacerbates iron overloading (through the increased gastrointestinal absorption), rendering iron

toxicity the principal cause of morbidity and mortality in transfusion-dependent or independent βTM patients. The life-saving transfusion therapy makes, as a side-effect, a secondary iron-overloading hit that worsens the already fragile and distorted host iron homeostasis. The deleterious effects of transfusional iron overload in βTM and SCD are demonstrated by increases in several markers of oxidative stress and inflammation, including lipid peroxidation [45,46], plasma protein carbonylation [47] and interleukin levels [48]. However, it must be noticed that a) the link between inflammation and transfusion has yet to be proved (see below) and b) there are clear differences between thalassaemia and SCD in respect to stress phenotypes [45]. For example, it has been reported that malondialdehyde and NTBI are less elevated in SCD than in βTM. In contrast, IL-6, IL–5, IL-10 and γ-tocopherol levels are higher in SCD [45,48].

Transfusion can exert a pro-oxidant/pro-inflammatory effect on the recipient by two ways: 1) directly, through administration of oxidized components, redox active free iron, haeme and Hb produced by in-bag haemolysis [49] and 2) indirectly, through the extravascular haemolysis of senescent or defected stored erythrocytes. In the second case, a swarm of surface modified RBCs may overwhelm the mononuclear phagocyte system of the recipient, inducing oxidative stress, cytokine secretion, susceptibility to infections and increased levels of NTBI [50]. Transfused critically ill children exhibiting substantial baseline haemolysis are characterized by increased NTBI, free haemoglobin and acute phase response as evidenced by elevation of C-reactive protein post-transfusion [51]. Since cell ageing, removal signaling and haemolysis are a function of storage age, older RBC units might be more effective in elevating NTBI levels and inflammatory responses in the recipient [52]. In premature infants for instance, transfusion with older RBCs induce increased levels of NTBI and pro-inflammatory cytokines [53]. However, blood age may not be directly related to post-transfusion haemolysis and inflammation [54] but instead, age can be overwhelmed by host and/or additional donor factors. To support, following blood transfusion in preterm newborns, a transient increase in NTBI was reported closely related with the age of blood and the elevation of oxidative stress, but not with the levels of pro-inflammatory cytokines [55]. In a recent study in transfusion dependent βTM children with suppressed erythropoiesis, serum ferritin and severe iron overload positively correlated to the serum hepcidin levels [43].

Taken together, the adverse effects of blood transfusion in paediatric patients with βTM and SCD seem to be a function of the pathophysiology of the disease in a context of developmental immaturity. As a result of transfusional iron overload, TDT children exhibit elevated lipid peroxidation, NOx, SOD and decreased GPx levels compared with age-and sex-matched non-anaemic healthy controls [56]. The management of iron overloading by chelation therapy is of even greater importance for the multi-transfused paediatric patients, because apart from the classic effects on cardiac and liver tissues, iron toxicity poses further risk of delayed sexual and physical maturation [57]. Interestingly, when compared with βTM subjects, the chronically transfused paediatric SCD patients exhibited similar levels of liver iron deposition but lower cardiac overload [58]. Finally, recipient variation and comorbidities still play a significant role, since transfused SCD children with underlying liver fibrosis or inflammation at biopsy demonstrate higher levels of liver iron overload [59].

4.2. Alloimmunization

As a major complication of transfusion, alloimmunization to RBC antigens is observed in all transfused populations. It comes of polymorphic immunogenic blood group antigens (e.g. *RH* allelles) that differ between donors and recipients. For instance, it is often the result of genetic and racial/ethnical disparity between SCD patients of African descent and donors of Northern European descent [60]. Antigenic differences are found within common variants and within variant antigens. In addition, some partial antigens can produce alloantibodies when exposed to the complete antigen.

Higher alloimmunization incidence has been reported in SCD compared with other patient populations. SCA further exhibits higher alloimmunization percentage than other SCD genotypes [21,22]. Children show lower alloimmunization compared with older patients (even after accounting for the number of RBC units transfused) [22], though, in chronically and episodically transfused children with SCD the prevalence of RBC alloimmunization is still high enough (7–29% [61,62] up to 45%). In only ABO/Rh-matched donor-recipient groups, alloimmunization ranges at 18–75% or 4-37% in SCD and thalassaemia, respectively [63,64]. Targeting to Rh and Kell comprise over 50–75% of the alloantibodies identified [65], followed by Kidd, Duffy, Lewis and MNS systems. Extended prophylactic matching for Rh, Kell and beyond has been associated with a marked decrease in the development of new antibodies in patients with SCD and thalassaemia [19,65].

Response to an individual alloantigen substantially increases the responsiveness (of paediatric/adult, and SCD/non-SCD patients) to additional, different alloantigens following a subsequent transfusion [60,66]. As recently shown in animal models, $CD4^+$ T cells sensitized to a RBC alloantigen are likely to directly augment a humoral response to a newly encountered and nontarget alloantigen cited on the same RBC [67]. Therefore, the unique characteristics of each antigen as well as the order of alloantigen exposure may influence the immunological outcome of transfusions. In addition, alloimmunization promotes autoantibody formation, that is higher in SCD (6–10%) compared with non-SCD populations [21,68], and in alloimmunized children with SCD on chronic RBC transfusion (69%) than on non-alloimmunized counterparts (11%) [61,62,68]. The pathophysiology that distinguishes alloantibody “responders” from “non-responders” is not clear, though it would be extremely useful for identifying susceptible individuals. There is evidence, that the percentage of memory $CD4^+$ T cells and the activity of regulatory T cells differ in responders compared with non-responders chronically transfused children and adults with SCD and βTM [62,69], while genetic variation in the Fcγ receptor gene was recently associated with lower risk for alloimmunization in SCD [70].

RBC phenotyping may be challenging in chronic or recent transfusions or when interfering antibodies and less common/variant alleles are present. Genotyping can provide improved accuracy and information for an extended matching that is particularly beneficial for past immunologic responders [66]. Following the molecular characterization of major RBC antigens, several genotyping assays have been developed in automated high-throughput instruments. The Food and Drug Administration approved one platform by which no phenotype confirmation with antisera is required [71]. The application of large-scale genotyping testing in routine practice, however, adds significant costs. Moreover, many matching protocols have failed to prevent alloimmunization [21]. Unfortunately, and despite progress in the field, the contemporary pediatric and adult patients with SCD are still characterized by a substantially higher rate of alloimmunization compared with the general population.

The risk of alloimmunization is further analogous to the number of exposures [21,61,62], with the majority of events being, however, observed after less than 15 transfusions [22,60]. As expected, in children with SCD the rate of alloimmunization also varies as a function of ethnic background [72]. Additional reported risk factors (though not verified in all patients under investigation) include patient age [21,22], age at first transfusion [73], recipient inflammatory state at time of transfusion, and the age of the RBC units [62,73,74]. A higher risk of alloimmunization has been reported in βTM patients first transfused at an older age [75], probably because the early initiation of transfusion program can induce tolerance. Nowadays there is a progressive decrease in age of initiation of transfusion and iron chelation therapies (even before the age of two), together with an increase in Hb values in children with transfusion-dependent thalassaemia [76]. In children with SCD, however, the age at initiation of chronic transfusion has not been universally associated with RBC alloimmunization [62].

Inflammation represents an established risk factor of alloimmunization following transfusion not only in SCD [74], which is a chronic inflammatory state *per se*, but in the general population as well [77,78]. Probably for this reason, episodic transfusions that are often performed in an inflammatory context have higher alloimmunization risk than chronic transfusions [61,73]. Despite the fact that erythrocytapheresis (ECP) requires increased blood exposure compared with simple transfusions, it has been associated with lower alloimmunization rates than simple chronic transfusions in paediatric SCD patients [65,79]. Removal of inflammatory cells and plasma by ECP [79] and the simultaneous exposure to a large number of antigens that may overwhelm the immune system, probably account for this differential effect [65]. Finally, female sex, a known risk factor for alloimmunization in the general population and in adult SCD patients, has not been widely reported as such in children [80]. When identified, the correlation is mainly with the increased number of transfusions in female compared with male groups and in part to pregnancy-associated alloimmunization in the age group of 16–20 years.

4.3. Hemolytic transfusion reactions (HTRs)

Blood transfusion in patients affected by βTM and SCD is accompanied by an appreciable rate of haemolytic transfusion reactions (HTRs) [40,63]. Destruction of donor RBCs in vivo not only abrogates the therapeutic outcome of transfusion but also leads to haemolysis-associated toxicity [81] and morbidity. The majority of the HTRs follow the development of RBC alloantibodies [71], however, antibody-negative HTRs can occur, particularly in the setting of SCD. Suppression of erythropoiesis, the genetic background of the donor (e.g. G6PD deficient donors in recipients receiving drugs, see below), mechanical RBC destruction during transfusion and the inflammation status of the recipient, may all fuel haemolytic pathways following a transfusion event.

Delayed hemolytic transfusion reaction (DHTR) is one of the most severe complications of transfusion in the paediatric SCD population, and often triggers VOC [82]. “Delayed” stands for the time of appearance, which is from 24 h up to three weeks (usually within 1 week) posttransfusion [83]. It manifests itself as acute haemolysis (> 25% drop in Hb levels) and clinical-laboratory features that resemble those of VOC, and hence, it may be misrecognized. Though basically observed in alloimmunization context with positive DAT, the immunohematology findings are often negative and new alloantibodies are not frequently detected, a fact that further contributes to underrecognition [40,82,84]. Consequently, a positive DAT is diagnostic (and the identification of the antibody should be performed) but a negative DAT does not rule out DHTR in SCD [83]. The high incidence of negative immunohematology findings in SCD DHTRs contrasts with most non-sickle DHTRs, suggesting different mechanisms of RBC destruction. Reticulocytopenia (compared to baseline) is paradoxically common in DHTRs, as a result of erythropoiesis suppression, iron deficiency, accelerated immunological destruction of reticulocytes, or of decreased erythropoietin levels secondary to kidney damage [40].

Delayed hemolytic transfusion reactions (DHTRs) are common in SCD, as being reported in 4–11% of patients transfused with apparently compatible blood transfusions [38]. This incidence mismatches alloimmunization one in SCD, when compared with the alloimmunization/DHTR events ratio in other transfusion settings, and as already mentioned, it may be rather underestimated. DHTR is most frequently observed in (but not restricted to) SCA compared to HbSC, probably in accordance with the frequency of transfusion therapy in the former.

Both diagnosis and treatment of DHTR can be extremely difficult and its prediction almost impossible. Under-diagnosis may lead to further transfusions that can prove fatal [85]. Similar to the risk of alloimmunization, the DHTR risk is higher after multiple transfusions and for those delivered in the acute compared with the elective, chronical setting in SCD patients [86]. Consequently, and following the increasing use of RBC transfusion therapies, the incidence of DHTR is expected to further increase in SCD patients.

In cases of alloimmunization-driven DHTR, either new formed alloantibodies or evanescent antibodies might occur. The latter, which have been found in all blood group systems, are not detected at screen or crossmatch, because their initial strength and titres reached by a previous transfusion are not high enough to keep them at detectable levels over time. However, a re-exposure to the antigen (by a new transfusion) may boost their production leading to DHTR. This phenomenon has been reported for almost half of the antibodies detected in SCD patients [22].

Since transfusion-induced immunomodulation may also result to temporary development of autoantibodies [87], severe DHTR can merge into autoimmune haemolysis, which is more common in SCD and usually requires treatment with long-term immunosuppression [21]. Successful prevention of DHTR through inhibition of development of autoantibodies secondary to transfusion has been achieved by specific targeting of circulating B cells prior to transfusion in SCD [88]. Surface modification of RBCs by alloantibodies might induce exposure of “neoantigens”, autoantibody formation and RBC lysis [89]. In fact, HTRs may be life-threatening if hyperhaemolysis, namely destruction of both donor and recipient RBCs [90,91], develops. In those cases, the Hb drops below the pre-transfusion levels. Though DHTR with hyperhaemolysis has been described mostly in patients with SCD, it is not uncommon in patients with βTM as well [85]. Hyperhaemolysis may or may not be antibody-mediated, since in only half of the cases DAT is positive and auto-/alloantibodies are present [40]. Like the antibody-negative HTRs, the mechanistic basis of DAT-negative hyperhaemolysis is unclear. Distinction between lysis of transfused versus autologous RBCs in SCD can be traced by Hb electrophoresis showing variation in patient (HbS) versus donor (HbA) Hb species.

Several antibody-dependent or independent hypotheses have attempted to explain RBC lysis in antibody-negative HTRs and DAT-negative hyperhaemolysis [83,84]. As previously mentioned, an antibody might be present at low levels, not detected by serology due to evanescence, subsequent development or retarded appearance in the serum. The detection techniques have variable sensitivity (e.g. agglutination vs. ELISA), especially for low-affinity antigens and certainly, the agglutination-based techniques are not capable of detecting all hemolytic antibodies. RBC lysis can be the result of bystander immune haemolysis [40], in which immunologic reactions to exogenous antigens injure innocent bystander RBCs. For instance, reactions to co-transfused antigens other than RBC-bound (e.g. HLA antigens and plasma proteins), may activate the complement to lyse RBCs [90]. Moreover, the immunogenicity of blood group antigens can be related to non-exposed polymorphisms (localized within the cytoplasmic or transmembrane domains of membrane proteins) that may promote production of RBC autoantibodies [89] and haemolysis [88].

A specific role of eryptosis and PS exposure in DHTR has been suggested by Chadebech et al. [92]. Specific, high-level of PS externalization is a prominent feature of SCD RBCs and VOC patients, pathophysiologically linked with anaemia, HbF percentage and prothrombotic reactivity [93]. Oxidative stress and binding of some antibodies (even at low-level) can trigger surface expression of PS on donor or autologous RBCs, if an autoantibody is implicated [94]. Of note, DHTR induces PS-exposure on RBCs in vivo. The prospective longitudinal study of Chadebech et al. [92] on adult SCD patients showed that the oxidative plasma from DHTR patients with no detectable antibodies induces PS phenotype and accelerated eryptosis of donor RBCs. Despite the fact that in those patients (for unknown reasons) the RBC lysis concerned only the donor and not the recipient SCD RBCs (that also exhibit increased PS exposure), lysis of donor RBCs may lead to a hemolysis-driven aggravation of the recipient’s clinical status, able to promote subsequent VOC and apoptotic death of autologous RBCs as well [92]. Moreover, hyperactive macrophages [81,94] can also hurt autologous RBCs through triggering VOC and non-immune reactions [86]. Other plasmatic factors with a probable influential role in this

mechanism are depletion of NO [95], extracellular vesicles, and certain inflammatory enzymes, such as the secretory phospholipase A_2 that can injure PS^+ RBCs [96]. Thus, lysis of donor RBCs may induce that of recipient RBCs as well; in other words, the DHTR can evolve to DHTR with hyperhaemolysis. Development of hyperhaemolysis may depend on variation in the expression of counteracting balancing "don't eat me" signals in host RBCs.

5. Special considerations for RBC transfusion in paediatrics

5.1. Deformability issues

In the early years of human life, the physiological properties of blood may affect its rheology. For instance, and besides the high haematocrit, both plasma viscosity and RBC aggregation levels are lower in neonates compared with the more mature stages, probably due to low plasma protein concentration [97]. The aggregability of the RBCs significantly increases 5–7 days after birth to adult levels [98], and blood viscosity increases from infancy to adulthood, despite remaining constant between the periods of early infancy to the second childhood [99]. In premature infants, the aggregability of RBCs is relatively higher, probably due to the bigger surface area and/or deformability of RBCs [100]. The deformability of neonatal RBCs is similar (slightly increased) to that of adult cells when studied under controlled conditions. In contrast, neonatal RBCs are less filterable [97], and impaired flow in narrow vessels (of diameter $< 3.3\,\mu m$) has been observed, owing to the large RBC size [100].

Thalassaemia major and SCD influence the rheological properties of RBCs as well, both in childhood and adulthood, since increased aggregation, lower deformability and enhanced adherence to endothelial cells have been reported [101,102]. Adherence to endothelium or deformability might be related to extracellular components (such as extracellular vesicles) or HbF/HbA_2 cell concentrations, respectively [102,103]. Focusing on children, decreased deformability in SCD correlates with lower cerebral tissue oxygenation index [104] and poor six-minute walk test performance [105]. Co-founders such as α-thalassaemia [106], iron overload and low levels of lactate dehydrogenase impact RBC deformability and were found related to sickle cell crisis [107]. In addition, RBC deformability, being at maximum levels during the early years of life in SCD, is strongly correlated with the HbF levels. During childhood, the HbF levels and the sex modulate RBC deformability in sickle patients independently of age, while blood viscosity is relatively higher in older SCD patients when compared with younger ones [108]. In fact, despite stabilization of HbF after 10 years of age, its levels remain independently associated with the RBC deformability in SCD patients [108].

Owing to the inherent association of SCD and βTM with RBC deformability issues, it is reasonable to assume that the rheological properties of transfused RBCs are relevant to the outcomes of transfusion in TDT and SCD patients. Storage impairs the biophysical and mechanical properties of RBCs, as evidenced by the reported low end-of-storage RBC deformability and the increased osmotic and mechanical fragilities, which are only partially reversed by rejuvenation [109]. In addition, the poorly deformable and susceptible to osmotic and mechanical stresses stored RBCs are prone to PS externalization [110]. Taken together, this branch of the RBC storage lesion might not be neutral in respect to blood rheology, adherence to endothelium and VOC episodes in susceptible patients. To support, transfusion is reportedly related to reduced blood perfusion and oxygen saturation in thalassaemic patients [111]. In the same group, post-transfusion increases in Hb [112] and skin blood flow [113] were found inversely correlated with the percentage of low deformable donor RBCs. Moreover, transfusion with stored RBCs of lower rigidity levels yields longer time intervals between two consecutive sessions [112]. However, in most cases, RBC transfusion to critically ill patients improves their blood circulation, as their RBC deformability is especially low [114]. Actually, skin blood flow was found related to the difference in deformability and adherence between transfused and recipient RBCs. Barshtein et al. reported that when the adherence/rigidity of stored RBCs is lower than those of the recipients' cells, the patient's blood flow is increased and vice versa [115].

5.2. Extracellular vesicles (EVs)

Patients with βTM and SCD are characterized by increased levels of plasma EVs, both at steady state and during sickle cell crisis, compared with normal subjects [116–118]. Along with chronic haemolysis, overproduction of EVs may be implicated in the pathogenesis of inflammatory and coagulation abnormalities encountered in both diseases [119]. Actually, by exposing procoagulant molecules, plasma EVs promote thrombin generation and increase the thrombotic risk in both health and numerous disease states [117,118,120,121], and there is accumulating evidence that they act as messengers between haemolysis and activation of haemostatic system [122]. In adult SCD patients, vesiculation is affected by splenectomy and various therapies [118], while the RBC-derived EVs have correlations with the number of circulating PS^+ RBCs and markers of haemolysis, coagulation and platelet/endothelial activation [118,121]. Moreover, the number of tissue factor positive EVs of monocyte and endothelial origin increase in sickle crisis versus steady state [117]. Thus, EVs are likely to represent bioeffectors involved in pathophysiological pathways, witnesses of cellular activation, and potential biomarkers for vascular dysfunction and disease severity, particularly useful in monitoring the response to pharmaceutical therapies [116].

In contrast to studies on adult patients [118,121], only limited data are available regarding the features of EVs in children with SCD. The vast majority of the available data have been collected by medium speed centrifugation of the plasma and flow cytometry approaches, thus, concerns EVs of larger size, commonly characterized as microvesicles (MVs) or microparticles (MPs). The MPs detected in infants and children with SCA originate mainly from platelets (PLTs) and RBCs [123–125]. The paediatric patients confirm the already known inverse relation between the concentrations of MPs and HbF [116,123,124], which modulates the severity of SCA and declines rapidly during the neonatal period. Consequently, and contrary to healthy children, in paediatric SCD patients the formation of EVs significantly increases with their age [124]. However, the HbF levels govern MP concentration by acting on specific MP subtypes [122]. Neonatal decline of HbF in older children (> 3 years) mainly coincides with increase in MPs derived from PLTs and monocytes and to a lesser extent from RBCs [123]. As expected, hydroxyurea treatment, is associated with a decrease in MPs of RBC and PLT origin [116,123].

In some cases, the MP levels have been found positively associated with the sickling crisis (compared with the steady-state), the pulmonary hypertension, markers of haemolysis, fibrinolysis, and iron overload, history of thrombosis or splenectomy, disease duration, transfusion index and HbS percentage [116]. Notably, while more PS^+ total MPs have been detected in children with SCA on transfusion therapy compared with those on sickle cell crisis, the MPs of RBC-, leukocyte- and endothelium-origin were found lower in transfused patients compared with those in crisis [126]. Finally, the plasma EVs vary between the several forms of SCD and the grade of SCA severity in paediatric patients. Actually, lower concertation of total MPs (mainly of RBC origin) was detected in HbSC children compared with those with SCA [125], and of note, the circulating exosomal microRNAs can distinguish the severe from the mild form of the disease in children with SCA [127]. Those EVs disrupt the endothelial barrier and induce monocyte adhesion on endothelial cells in culture, in a SCA severity-dependent manner.

Plasma EV levels have been repeatedly found higher in children and young patients with βTM compared with healthy controls, as well [116,128]. Moreover, they have been reported higher in thalassaemic

patients who experienced thromboembolic events compared with those without events, suggesting a predictive role as risk factors. In young patients with βTM, MPs generation has been also found closely related to tissue hypoxia, oxidative stress, splenectomy, markers of haemolysis, iron overload, D-dimer, vWF antigen and βTM-associated comorbidities, including aortic stiffness and pulmonary hypertension [129]. However, it has been suggested that only the PLT-derived MPs contribute to the thrombotic risk in splenectomized βTM patients (age of 17–58 years). Splenectomy promotes PLT-vesiculation and the phospholipid-dependent procoagulant activity, because the counterpart activity of RBC MPs is diluted by transfusions. Actually, the RBC-derived MPs originate only partially from the thalassaemic erythropoiesis (HbF^+) [128]. Just as observed in SCD paediatric patients on hydroxyurea therapy, compliant paediatric patients with βTM on chelation therapy had lower MP levels than their non-compliant counterparts [129]. Moreover, decreased levels of RBC MPs were detected in young βTM patients after bone marrow transplantation compared with those who received regular blood transfusions [130]. Considering that the standard RBC units have extremely low concentration of NOx and progressively accumulate free Hb and Hb-containing EVs (both scavengers of NOx), there is the theoretical (at least) possibility that not only the older RBC units, but also those with comparatively higher levels of in-bag haemolysis and EVs may exert appreciable vascular effects to susceptible SCD and βTM patients.

5.3. Age of blood

There is evidence that autologous transfusion of RBCs after prolonged storage (6 weeks) to healthy adults is associated with extravascular haemolysis and decreased recovery [131]. A much shorter storage age of transfused RBCs (up to 3 weeks) correlates with increase in NTBI following transfusion in very low birth weight infants [132], suggesting that some paediatric patients are potentially more susceptible to the deleterious effects of older blood. No doubt, those adverse events might negatively impact episodic or chronic transfusion therapy outcomes in SCD and thalassaemia. Fasano et al. [74] that retrospectively studied a cohort of alloimmunized SCD patients, reported an association between the age of blood and the alloimmunization risk, but they did not reach a definite conclusion on this effect, secondary to sample size concerns. On the opposite side stands a recent retrospective cohort study that did not find any significant relationship between the age of RBCs (with a median, however, duration of "old" units on 17 days) and hospitalization length or oxygen requirements in young adults and children ($\leq$22y) with SCD and ACS treated with simple RBC transfusion [133]. In the same direction, the concurrent TOTAL randomized clinical trial found that RBCs of longer-storage correct lactic acidosis and increase cerebral tissue oxygen saturation as effectively as shorter-storage RBCs in paediatric patients with SCD and malaria [134].

The current transfusion guidelines bring together guidance for adults and children with SCD or thalassaemia [16]. In spite of limited literature supporting any beneficial effect of fresh RBCs, it is common practice to provide certain high-risk populations, such as neonates and patients with SCD and βTM, with the "younger" available RBC units ($<$ 14 days) [135]. If the transfusion community feels a need to change this practice, then prospective studies are warranted to examine the clinical implications (if any) of age of blood in transfused paediatric patients with SCD and thalassaemia. However, we should keep in mind that storage time is only one (rather than the most prominent) of the factors affecting the quality of labile blood product.

5.4. Donor and recipient variation

Analysis of donor and recipient biology by high throughput state-of-the-art omics and other novel technologies (e.g. nanotechnologies or contemporary microscopy), integration of the complex results in multi-omics or omics/physiological data platforms, and their channeling to system biology models have both updated and broaden the research field in transfusion medicine [136]. In this context, a re-evaluation of age of blood has occurred, new concepts emerged, and several underestimated factors introduced themselves as standing hierarchically above the age of stored blood in respect to the progression of storage lesions and the post-transfusion performance. Among these, the metabolic ageing of stored RBCs and the physiological/genetic background of donors and recipients are included [137,138]. Paediatric patients with SCD and thalassaemia in need of transfusion therapy represent a very sensitive recipient group, currently (though, informally) treated by the "best available blood", which is considered to be (among other properties) the "younger" one RBC unit. However, in the light of recent advances in the assessment of the storage lesion, this common practice seems rather outdated and probably not so effective as formerly thought.

Officially, the World Health Organization guidelines categorize neonates and infants as a susceptible recipient group for matching with G6PD-deficient donors [16]. Data collected from in vitro models of transfusion [139] showed that stored RBCs from G6PD deficient donors exhibit increased haemolysis and oxidative lesions compared to control RBCs when found in transfusion-mimicking conditions, in the absence of any pathological or disease setting. According to clinical reports, transfusion of G6PD-deficient blood may have adverse effects on premature neonates, infants, recipients with G6PD deficiency themselves, and regularly transfused patients [140]. As expected, more deleterious effects have been reported on neonates and children than on adult susceptible patients. These findings come to certain self-evident conclusions: a) the outcome of each transfusion is actually the result of the critical interplay between donor and recipient characteristics, and b) the small age of the recipient is a conditional risk factor for negative clinical phenotypes following a transfusion. A recent study in children with SCD on chronic transfusion therapy identified some donor and host participants in this interplay: G6PD deficiency at donor side, as well as splenectomy and RBC alloimmunization history at SCD recipient side seemed to affect the survival of transfused RBCs in vivo and the suppression of sickle erythropoiesis [141], in a risky way that promotes cerebrovascular disease and stroke despite chronic transfusions [142]. Notably, we have recently detected by metabolomics analysis of RBC transfusates different levels of phthalate plasticizers and breakdown products in RBC units from G6PD deficient donors compared with the control [143]. Blood donors are not routinely screened for G6PD deficiency and besides, its prevalence among randomly selected RBC units is very low (0.3%) [144]. However, this is not the case for the transfusion setting of SCD patients: there is a much higher prevalence (12.3–15%) of G6PD deficiency (and Hb variants) among RBC units selected for SCD patients [141], due to increased antigen matching requirements and the racial characteristics of blood group antigen distribution [145]. Therefore, selection against G6PD deficient RBC units may be a consideration for SCD patients.

Apart from G6PD deficiency, (and for currently unknown reasons), collection and leukoreduction of blood from donors with sickle cell trait pose also some challenges. Actually, failure of the filtration process occurs more often [146] and susceptibility to haemolysis during the procedure is higher in those collections [147] compared with the control ones. Though not shown to reduce morbidity or mortality, use of sickle-negative blood has been recommended for SCD patients and is provided at most comprehensive SCD centers that perform universal leukoreduction [135]. No recommendations exist for donors with other Hb variants, despite the fact that the physiological properties of RBCs in thalassaemia traits for instance, could be relevant to the progression of storage lesion [148]. The field lacks studies like this and we can only speculate about the donor/recipient matching outcomes. Therefore, further research on the adequacy of healthy donors with G6PD deficiency and other enzymopathies, as well as with various Hb variants (e.g. beta thalassaemia trait) for transfusion in paediatric patients on transfusion therapy is of great value.

5.5. Blood bag plasticizers

Phthalates, like the Di(2-ethylhexyl) phthalate (DEHP), are plasticizers that offer significant mechanical and biological properties (including durability, flexibility, compatibility and gas exchange) to vinyl plastics [149]. DEHP is non-chemically bound to the plastic and thus, it can readily leach out of it. In blood storage setting, a significant part of this leakage tends to incorporate into the membrane and cytosol of stored RBCs [150], and by doing so, it reduces the RBC storage lesion [150,151] and improves the RBC recovery [151]. In contrast, DEHP and its metabolite monoethlyhexyl phthalate (MEHP) promote the release of IL-8 from human endothelial cells in vitro, suggesting a proinflammatory potential [152]. The levels of DEHP and its metabolites in-bag increase with storage time [152,153] and vary depending on the temperature and blood component, with the highest levels being reported in whole blood and then, in RBC concentrates [153]. Subsequently, the soluble or RBC-associated phthalates are infused along with the transfusate to the recipients. However, as potential toxins, they may have harmful effects to them [154]. Reproductive toxicity, hepatotoxicity, teratogenicity, hepatic and renal malignancies and alterations in cardiovascular reactivity are among the adverse effects shown in animal models [155]. In humans, DEHP is classified as an endocrine disrupting compound [156]. Finally, according to a recent longitudinal study in mother-child pairs, early life phthalate exposure is associated with decreased thyroid hormone levels in young children [157].

In the light of those mixed effects, elimination of DEHP from medical devices, including blood bags, is the subject of intent debate [158]. Concerns have been mostly raised for vulnerable populations, such as medically exposed (through both medical devices and transfusion) pregnant women, neonates, infants and young children, mostly males, since the developing male reproductive tract appears to be particularly susceptible to damage [154]. Though exposure by blood transfusion may be lower than toxicity levels and only a small proportion of the daily exposure by food storage and common household products, DEHP intake by ingestion differs substantially from intravenous intake through transfusion, because its breakdown is much lower into the blood stream than in the gut. The degree of transfusional exposure to DEHP is a function of transfusion frequency, volumes of blood products and processing (e.g. leukoreduction, irradiation). Thus, it can potentially exceed the recommended tolerable daily intake levels in at-risk groups, such as transfusion-dependent paediatric patients. Several regulatory agencies have highlighted increased risk of toxicity due to DEHP in blood bags, particularly when high-volume transfusions occur, such as neonatal RBC exchange and massive transfusion [159].

Early quantitative studies on exposure of newborn infants to transfusional DEHP and MEHP raised serious concerns about the pharmacokinetics of these potentially toxic metabolites [160]. Higher urinary concentration has been later reported in adult patients receiving blood transfusion than in non-transfused hospitalized patients receiving other medical care [161]. In similarity to those adult groups, urine levels of DEHP and its oxidative metabolites in premature infants who underwent a wide variety of medical procedures (including blood transfusion) is reported to be much higher compared with those of a reference population of healthy children > 6 years old [162].

As for other transfusion-associated risks, phthalate exposure may be more toxic for fetuses and neonates than for more mature individuals. At earlier developmental stages, the absorption/excretion, distribution and metabolism of incoming DEHP may differ substantially [149]. The intravascular circulation of DEHP, for instance, is expected to be longer in neonates, because the metabolizing capacities of liver, intestine, lung and blood (needed to convert DEHP to MEHP) are not completely active until the 6 months of age. Its distribution in the developing gonads and central nervous system may be also higher due to the immaturity of the blood-brain and blood-testis barriers. Moreover, the necessary physiological machinery for the excretion of phthalates (e.g. renal capacity) is not completely developed in those young individuals.

Several alternative plasticizers have been suggested as promising candidate substitutes for DEHP [163]. They have been evaluated in respect to leakage rate, in-bag haemolysis, RBC metabolism, potassium release, osmotic fragility and EVs formation. The quality of RBC concentrates stored in paediatric bags formulated with alternative plasticizers to DEHP was also investigated [164]. It has been suggested that the blood bank community should again explore alternative plastics to protect highly vulnerable populations exposed to blood transfusion.

6. Future perspectives

In the emerging era of personalized transfusion medicine, paediatric patients on transfusion therapy represent a recipient group of outstanding interest. The well-characterized, distinct pathophysiology and special needs for transfusion therapy, mostly arisen by the developmental immaturity, can and should be used as a “compass” to guide our steps toward a better understanding of donor/recipient variation effects on transfusion outcomes, which will confidently put critical pieces of the unsolved matching puzzles together. Widespread molecular testing of donors and recipients by high-throughput genotyping and molecular characterization in the future may lead in a more individualized approach to transfusion medicine [83]. Apart from RBC antigen matching, the ongoing increasing application of new technologies and scientific advances into the field of blood storage and transfusion biology, has resulted in a milieu of additional candidate factors and potential indicators of good or poor performance, whose almost infinite combinations render identification of the “optimal matching” a riddle [136]. Characterization of a patient group at genetic level reduces (but not eliminates) the recipient-to-recipient coefficient variation and consequently, the “pool” of donor-specific blood that most appropriately fits an effective and low-risk personalized transfusion. Transfusion therapy in paediatric patients with βTM and SCD would be markedly benefited by future research initiatives on: a) clinical and genetic factors functionally associated with the susceptibility to alloimmunization; b) factors underlying RBC destruction in antibody-negative HTRs and DAT-negative hyperhaemolysis; c) the variation in bioreactive EVs as a function of donor features, blood preparation methods and clinical outcomes (e.g. VOC, tissue hypoxia etc); d) the exact role of disease- and transfusion-driven oxidative stress in the long-term progress of the disease by using oxy-omics technologies; f) the contribution of donor RBC biophysical and biomechanical features to vascular host responses and extravascular haemolysis; g) the examination of donors with enzymopathies and Hb variants for crossing with paediatric patients; h) new randomized clinical trials to elucidate the age of blood effects on paediatrics, based on “extended” age appreciation and other metrics; and i) alternative, less toxic plasticizers, dedicated for usage in sensitive paediatric populations in need of hospitalization, medical care and transfusion.

Conflicts of interest

None.

Transparency document

The Transparency document associated with this article can be found in the online version.

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