Challenges in chronic transfusion for patients with thalassemia

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The introduction of regular red cell transfusions 60 years ago transformed β-thalassemia major from a fatal childhood illness into a chronic disorder. Further advances in the prevention of transfusion-transmitted infections and management of iron overload have allowed survival and quality of life to approach normal. However, transfusion therapy for some other thalassemia syndromes continues to challenge clinical decision-making. Nearly one-half of the patients with E ß thalassemia are transfusion-dependent, yet the criteria for initiating transfusions or hemoglobin targets are not well defined. Patients with thalassemia intermedia who begin transfusions as adults are at very high risk for developing red cell alloimmunization and serious hemolytic transfusion reactions. In the growing number of survivors of Bart hydrops fetalis, the approach to transfusion therapy and iron chelation is rapidly evolving. A collaboration between hematology and transfusion medicine specialists will be essential to improving patient care and developing evidence-based guidelines.

LEARNING OBJECTIVES
- Recognize the heterogeneity of transfusion-dependent thalassemia and its impact on management
- Understand barriers to the effective use of red cell transfusions in thalassemia intermedia, hemoglobin E beta thalassemia, and alpha thalassemia major.

In the past decade, the classification of patients into transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT) was widely adopted. These terms were beneficial in planning the management of iron overload or choosing stem cell transplant or other curative therapy based upon a patient's transfusion status. However, this approach can conceal the tremendous heterogeneity of TDT, a phenotypic group that encompasses β-thalassemia major, severe β-thalassemia intermedia, hemoglobin (Hb) E (HbE) β thalassemia, and certain α-thalassemia syndromes. Among these, β-thalassemia major is the largest category and is usually associated with the presence of 2 severe β-globin mutations.[1] These infants become symptomatic from anemia within the first year and regular transfusions are instituted before 2 years of age.[2] The natural history of β-thalassemia major has been the best characterized among various entities constituting TDT, and, consequently, the transfusion guidelines recommended by various groups for β-thalassemia major are largely similar.[1,3-8]

Heterogeneity of TDT
The guidelines developed for β-thalassemia major may not be appropriate in managing the other thalassemia syndromes that require regular transfusions. The severity of β thalassemia is determined by the imbalance between the α and non-α globin chains. A reduced or complete absence of β-globin synthesis leads to accumulation of excess α-globin chains that are toxic to the erythroid precursors.[9,10] The surplus of α-globin chains can be mitigated when 1 or both β-thalassemia alleles are mild (β+ or β++), there is concurrent deletion of α-globin genes, or elevated synthesis of γ-globin persists.[9] Elevated γ-globin synthesis sometimes arises from hereditary persistence of fetal Hb, but milder increases are more often from quantitative trait loci in *Xmn1-HBG2*, *HMIP*, and *BCL11A*.[11] Various forms of β thalassemia may also differ in the total endogenous Hb (F, A, or E) or the oxygen-affinity characteristics (A and E compared with F)[12] that modify the adaptation to anemia.[13] Individuals with severe forms of α thalassemia, in contrast, produce nonfunctional Hb (Hb Bart or HbH), which causes underestimation of the true severity of anemia.[14] In β-thalassemia intermedia and HbE β thalassemia, the decision to commence regular transfusions is influenced not only by the severity of symptoms, but also on medical judgement. The latter is a subjective assessment of whether long-term prognosis would be

better by accepting symptomatic anemia without transfusions instead of transfusion dependence and the associated potential complications. The recognition that patients with NTDT have worse quality of life than those with TDT and are at risk for severe complications has led to the extension of chronic transfusions to a larger proportion of patients than in the past.[15-18]

The role of regular transfusions in HbE β thalassemia

HbE β thalassemia is caused by compound heterozygosity for the E mutation (HBB:c.79G>A) and a β-thalassemia mutation.[19] The prevalence of HbE β thalassemia follows the distribution of the E mutation, which reaches very high frequencies in southeast Asia, southern China, and south Asia. Immigration from Asia to the west has increased the awareness of this syndrome and its distinctive natural history compared with β-thalassemia syndromes (caused by 2 β-thalassemia mutations).[20] The severity of HbE β thalassemia ranges from a mild, asymptomatic anemia to the development of transfusion dependence from early life.[19] The E mutation activates a cryptic splice site that reduces synthesis of β^E messenger RNA.[21] The variable decrease in β^E output is 1 of the factors underlying the variable disease phenotype, even though HbE is a functional Hb. The severity of β mutation (β^+ instead of β^0), coinheritance of α-thalassemia trait, and genetic traits that increase γ-globin synthesis reduce the severity of HbE β thalassemia.[19] Why patients may have dissimilar physiological response to nearly identical Hb levels, and why erythropoietin response to anemia declines with age, is incompletely understood.[22] One characteristic that differentiates HbE β thalassemia from β thalassemia (intermedia or major) is the different functional properties of HbE and HbF. Patients with HbE β thalassemia compensate by rightward shift in the oxygen affinity, which is not seen in β thalassemia where the HbF is the predominant Hb.[12] Although clinical symptoms increase progressively with severity of anemia, it may not be possible to predict the likelihood of transfusion dependence based on Hb concentration alone.

Case 1

The patient was diagnosed with HbE β^0 thalassemia based on results of newborn screening. She was asymptomatic in early childhood with no limitation of physical activity, mild facial skeletal changes, and normal growth. Her Hb concentration was maintained between 6.7 and 7.1 g/dL without any blood transfusion. At 7 years of age, the spleen started to enlarge from 3 to 6.5 cm along with a decline in height velocity. She started regular blood transfusion at 10 years of age that led to resumption of normal growth and a decrease in spleen size to 2 cm (Figure 1).

Case 2

The patient was diagnosed with E β^0 thalassemia at 3 years and started regular transfusions with iron chelation. He was splenectomized at 12 years due to higher blood requirements and splenomegaly, following which Hb was spontaneously maintained between 6.9 to 7.2 g/dL. He was transfused intermittently 1 to 2 times per year when Hb dropped below 7 g/dL. In his early 20s, he developed dyspnea and continuous oxygen requirement from severe pulmonary arterial hypertension and heart failure. He had severe iron overload with liver iron concentration of 38 mg/g and cardiac magnetic resonance imaging T2* of 4.9 ms. Heart failure and pulmonary hypertension

improved with supportive management, regular transfusions, and iron chelation (Figure 2).

Discussion

As expected for β-globin disorders, newborns with HbE β thalassemia do not develop anemia until the synthesis of HbF declines significantly over the first 6 months of life. Some infants display the classic symptoms observed in β-thalassemia major, including failure to thrive, hepatosplenomegaly, pallor, and fatigue.[23] More often, the symptoms are mild and escape attention until an incidental viral infection or a routine blood test reveals anemia. In California, where universal newborn screening is practiced for thalassemia, observations suggest that normal growth and development persist up to 1 year and beyond in many, but not all, infants with HbE β thalassemia. When the diagnosis is made later during life due to pallor, anemia, facial skeletal changes, growth failure, hepatosplenomegaly, or jaundice, the age at presentation is an important marker of the severity of disease.[24]

Decisions on appropriate timing to either initiate or discontinue chronic transfusion therapy, although difficult, are of principal importance to the management of HbE β thalassemia.[22] Patients with baseline Hb <6 g/dL should be placed on transfusions even when asymptomatic. Conversely, it is unlikely that patients with baseline Hb >8 g/dL would benefit from transfusions. Finally, those with Hb between 6 and 8 g/dL should be evaluated according to the proposed guidelines (Table 1). Our practice is to evaluate children with HbE β thalassemia in the clinic every 3 months for careful assessment of growth, splenomegaly, facial skeletal changes, and Hb level. Electronic medical records are useful to review trends over time and to store photographs for assessment of bony changes.

In our practice, splenectomy is discouraged as a strategy to avoid the need for transfusions because the effect on Hb may be short-term and it does not address the underlying severe pathophysiology.[22,25] On the contrary, splenectomy increases the risk of infections and a number of serious long-term complications such as thromboembolism, pulmonary hypertension, and iron-induced endocrinopathies.[25] Other avenues to improve anemia include hydroxyurea, which produces an ~1 g/dL rise in total Hb in 50% of patients,[26-28] however, the response in HbE β
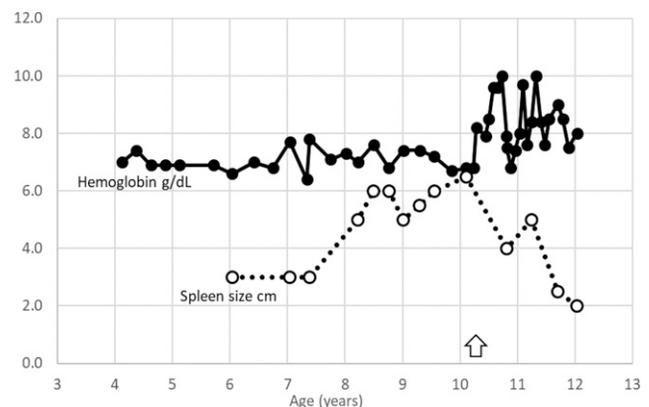
Figure 1. Case 1 depiction. In a patient with HbE β thalassemia (case 1), progressive increase in spleen size and reduced growth velocity led to start of regular transfusions (arrow). Transfusions led to reduction in splenomegaly over the following 2 years.
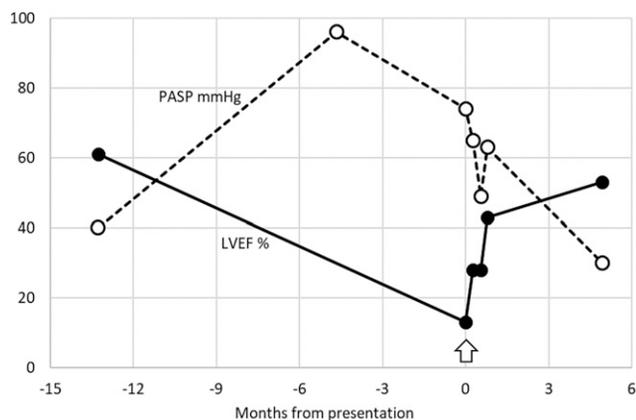
Figure 2. Case 2 depiction. In a transfusion-dependent patient with HbE β thalassemia (case 2), splenectomy was followed by discontinuation of regular transfusions. Fifteen years later, severe pulmonary arterial hypertension and congestive heart failure developed. These pathological changes were reversed by resumption of transfusions (arrow) and supportive care. LVEF %, left ventricular ejection fraction (percent); PASP, pulmonary artery systolic pressure.

thalassemia is variable and insufficient to eliminate the need for transfusions.[18] Luspatercept is an approved therapy to reduce the transfusion requirements in patients with TDT including HbE β thalassemia.[29] Results of a phase 2, open-label study of luspatercept in NTDT showed ≥1.5 g/dL improvement in Hb in 14 of 31 subjects,[30] but the pivotal trial has not yet been done. Mitapivat, a pyruvate kinase activator in the red cells, is also being evaluated in NTDT.[31]

Case 2 highlights the underrecognition of iron overload in patients with NTDT.[18,32] Patients who receive only intermittent transfusions can develop marked iron overload over time. Gastrointestinal absorption of iron is increased in nontransfused patients due to hepcidin insufficiency induced by ineffective erythropoiesis and elevated erythroferrone.[33-35] Screening for iron overload is recommended in all patients with NTDT irrespective of transfusion history. Although magnetic resonance imaging for assessment of liver iron concentration is more accurate,[36] we consider serum ferritin to be useful as a screening test for iron overload. Serum ferritin values underestimate the liver iron overload in NTDT compared with TDT, therefore, LIC should be measured when ferritin exceeds 300 ng/mL. Iron chelation is indicated if LIC is >5 mg/g liver weight[36] with deferasirox at a starting dose of 3.5 to 7 mg/kg per day (5-10 mg/kg if the dispersible tablet is used).[37] We evaluate liver iron concentration every 6 months during therapy and stop chelation therapy when LIC <3 mg/g is achieved.

Red cell alloimmunization in patients starting transfusions as adults

The development of antibodies to red cell antigens is a significant threat to the long-term success of transfusion therapy.[38-43] In the United States, the proportion of patients with thalassemia with red cell alloimmunization is 17% to 22%, only slightly lower than sickle cell disease (19% to 31%).[38,44,45] Older age at initiation of transfusions and splenectomy are identified as major risk factors for developing alloimmunization in thalassemia.[38,41,46] The

specificity of antibody and the risk of alloimmunization is influenced by the disparity in red cell antigens among different ethnic groups.[47] In countries where thalassemia predominantly affects immigrant communities, the greater degree of donor-recipient red cell antigen mismatch can elevate the risk of alloimmunization, as shown in Table 2.[39,44,46,48-50] In particular, the low frequency of Kell and c antigens in patients of Asian background facilitates development of alloimmunization.[39,46] One-half of the patients with 1 alloantibody will develop further antibodies against 1 or more additional antigens.[44] Transfusions can become progressively more difficult and it may become nearly impossible to find matched units for certain patients. The most frequent antibodies are against Rh and Kell groups,[41,44] which implies that universal phenotypic matching for these antigens can be a cost-effective method to prevent development of most red cell antibodies in thalassemia.[41,46]

Case 3

A 50-year-old woman with β-thalassemia intermedia who had undergone splenectomy recently changed hospitals. She was diagnosed at the age of 30 years but was only transfused during pregnancy. Due to worsening fatigue, regular transfusions were recommended by her new hematology team. A red cell phenotype was checked, and an antibody screen was found to be negative. She was given 2 red cell units matched to c, E, and Kell antigens. Later that night, she developed chills and fever. Her Hb levels were 7.1 and 9.0 g/dL, respectively, before and after the transfusion. Laboratory testing showed that her lactate dehydrogenase was normal, bilirubin rose from 1.2 to 1.7 mg/dL, haptoglobin was low, and urine contained trace Hb. Two weeks later, the antibody screen was positive and anti-Fyb was identified. Historical antibody data obtained from the previous

Table 1. Indications to begin chronic transfusion therapy in HbE β thalassemia

Indications
Hb <6 g/dL at baseline
Hb 6-8 g/dL accompanied by symptoms
• Growth
− Infants (<2 y): failure to gain weight for 3 mo
− Children: Height velocity <3 cm/y
− Older children: Delay in puberty: >12 y in females, >13 y in males, endocrine evaluation
• Skeletal facial changes: subjective, discuss with patient and family
• Splenomegaly: Spleen >6 cm, or enlargement >1 cm/y after 2 y of age
• Extramedullary hematopoiesis: symptomatic or moderate to large masses
• Cerebrovascular: overt stroke, silent infarcts, arterial narrowing, moyamoya
• Venous thromboembolism
• Pulmonary hypertension
• Osteoporotic fracture
• Quality of life in adults: decline in capacity to work or perform usual activities

Table 2. RBC antigen frequencies and prevalence of alloantibodies

Antigen	White, %	Black, %	Chinese, %	Asian Indian, %	Lal et al, 2018,[39] n = 314 pts	Singer et al, 2000,[46] n = 64 pts	Thompson et al, 2011,[38] n = 697 pts
D	85	92	99	94	1		4
C	68	27	93	87	1		11
C	80	96	47	58	4	2	7
E	29	22	39	20	8	4	22
E	98	98	96	98	2		7
K	9	2	0	3.5	15	6	21
K	99.8	100	100	100			
Fy^a	66	10	99	87	2		2*
Fy^b	83	23	9.2	58	1		
Jk^a	77	92	73	81	4		9†
Jk^b	74	49	76	68	2	1	
M	78	74	79.7	89	1	1	
N	72	75	67.4	65	1		
S	55	31	8.7	55	3		3
S	89	93	100	89			

RBC antigen frequencies among ethnic groups[48-50] and prevalence of significant alloantibodies among patients with TDT in the western region of the United States,[39,46] and North America and United Kingdom.[38]
pts, patients.
*Fy^a or Fy^b.
†Jk^a or Jk^b.

blood bank showed anti-c, anti-E, anti-Kell, and anti-Fyb antibodies had been previously identified 15 years ago. The patient was successfully maintained on regular transfusions with extended phenotypically matched red blood cells. Anti-Fyb could no longer be identified after 4 years, although her antibody screen was intermittently positive due to development of anti-Cw antibody (Figure 3).

Discussion

Although considered a much greater risk in sickle cell disease,[51] delayed hemolytic transfusion reactions (DHTRs) are also observed in thalassemia.[52] Hemolytic transfusion reactions have been reported due to anti-E, anti-Jk^b, anti-Jk^a, anti-c, anti-S, anti-Kell, and anti-f.[39] More than 25% of older children and adults with thalassemia will develop an alloantibody following 1 or more transfusions when red cell matching is limited to ABO/D only.[41,44,46] In the absence of further antigenic exposure, one-third of alloantibodies become undetectable within the first year of follow up. Anti-Jka antibodies are very evanescent, falling below the limit of detection within the first month of initial detection, whereas the anti-Kell and anti-E antibodies are undetectable in >50% at 6 months.[53,54] Between 20% and 25% of individuals of Chinese and Asian Indian ethnicity are Jka−, 60% to 80% are E−, and virtually all are Kell−.[48,55] An additional concern is c antigen which is negative in 40% to 50% of Asian patients, but the evanescence rate for anti-c antibody is lower (25%) compared with the other alloantibodies.[39]

Sensitized patients who receive a later transfusion, based on a negative antibody screen, rapidly increase antibody titer with reexposure that develops into a DHTR of variable severity.[51] DHTRs are less likely in regularly transfused patients where antibody screening is performed every few weeks and newly developed antibodies are unlikely to become undetectable before the next transfusion.[56] However, low-titer antibodies may be missed, or antibodies may develop while donor red cells are still circulating in significant amounts, leading to hemolysis. Intermittently transfused patients, on the other hand, are at high risk for antibody evanescence between transfusion episodes. The number of such patients with thalassemia, transfused 1 to 6 times per year, is small in the United States but significant in regions where blood availability is limited. Infrequent transfusions may be recommended during an infection, surgery, or
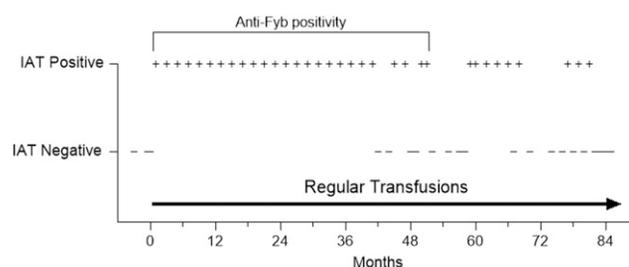
Figure 3. Case 3 depiction. In a patient with β-thalassemia intermedia with prior exposure to blood and negative antibody screen (case 3), the transfusion of red cell units matched only to Rh and Kell antigens led to the reemergence of anti-Fyb antibody and delayed hemolytic transfusion reaction. Regular transfusions were possible with extended phenotypic matching of red cell units. Anti-Fyb was no longer detectable after 4 years, though antibody screen remained positive intermittently due to other alloantibodies. IAT, indirect antiglobulin test.

pregnancy.[25] Transfusions during pregnancy are associated with a very high risk of alloimmunization, but these antibodies decrease in titer when transfusions are discontinued after childbirth.[57] Patient transfer between hospitals poses a persistent challenge to transfusion safety.[58] The lack of comprehensive antibody history can easily lead to the transfusion of red cells to which the patient is previously sensitized but now has a negative antibody screen. Although attempts have been made to provide patients with transfusion cards that list their phenotype and red cell antibodies, their use remains erratic. The absence of a centralized database for multiply transfused patients remains a serious shortcoming of the current practice of chronic transfusion therapy.[59]

Postnatal management of Bart hydrops fetalis: ATM
The deletion of all 4 α-globin genes (–/–, homozygous α[0] thalassemia, or α-thalassemia major [ATM]) causes severe fetal anemia.[14,60] As fetal viability depends upon the preservation of embryonic ζ genes (ζ-/ζ-, or –/ζ-), the most frequent α[0] deletion associated with ATM is the southeast Asian deletion (–SEA).[14] The major Hb species in ATM is Hb Bart (γ_4), formed by self-association of γ chains into tetramers in the absence of α chains. Because Hb Bart is ineffective in transporting oxygen, most pregnancies end in fetal demise following a variable period of hydrops (Bart hydrops fetalis).[60] Intrauterine transfusions (IUTs) are essential for the fetus to reach viability with acceptable neonatal outcome.[61,62] All infants with ATM are transfusion-dependent from birth and require recognition of the nonfunctional Hb fractions for correct management.[62,63]

Case 4
A child was born to parents who were carriers of the –SEA deletion and had previously experienced a hydrops-associated stillbirth. During this pregnancy, fetal hydrops was detected at 20 weeks of gestation, and managed by IUT performed on 6 occasions. The baby, born at 37 weeks weighing 3.0 kg, was stable and underwent a red blood cell exchange transfusion at 48 hours. Following discharge, he started regular red cell transfusions every 4 weeks. The average pretransfusion Hb was 9.7 g/dL with Hb Bart and HbH accounting for 20% to 36% of the total value. Starting at 8 months, the transfusion regimen was changed to maintain HbH <20%, which corresponded to HbA >9.0 g/dL and total Hb of ~11 g/dL in the pretransfusion blood sample. Growth proceeded at the normal pace, and developmental assessment at 3 years showed age-appropriate attainment of milestones (Figure 4).

Discussion
The management of ATM is evolving with experience gained from data in international registries[61] and publication of case series.[62-65] In the absence of existing consensus guidelines, our institutional practices are provided in this discussion to fill gaps in published literature. Questions about the optimal management of pregnancies affected by ATM are being evaluated in an ongoing clinical trial (NCT02986698, fetus.ucsf.edu).

The hematological management of ATM commences as soon as the diagnosis is suspected during pregnancy. In that small proportion of cases in which both parents are known carriers of the α[0]-thalassemia trait, the diagnosis should be established expeditiously with DNA testing from chorionic villus biopsy instead of ultrasound surveillance for fetal changes suggestive of

hydrops. In most cases, however, the first indication is the detection of hydrops on ultrasound during the second trimester. In such cases, a presumptive diagnosis of ATM is appropriate when severe fetal anemia is observed by Doppler ultrasonography,[66] and the pregnant woman is not alloimmunized to RhD or other red cell antigens but has microcytosis and hypochromia. Hematologists have a critical role to play in confirmation of the diagnosis, nondirective counseling of the family, and prenatal management of the fetus.

When the decision is made to continue the pregnancy, the first IUT should be initiated as soon as possible. The strategy for conducting IUT in ATM is derived from consensus guidelines from the Society for Maternal-Fetal Medicine.[67] The decision to proceed with fetal blood sampling and IUT should be based on the detection of severe fetal anemia (defined as elevated peak systolic velocity in the fetal middle cerebral artery on Doppler ultrasonography[68]) irrespective of the presence of hydrops.[67] Because Hb Bart, which constitutes nearly all the Hb in ATM, does not participate in oxygen transport, fetal hypoxia is disproportionate to any given Hb level.[69] The goals of complete correction of anemia or suppression of fetal erythropoiesis must be balanced against the risk of acute cardiovascular alterations and hyperviscosity with IUT.[67] Fetal anemia develops early in ATM with a mean Hb of 6.8 g/dL at 18 weeks,[69] which implies that there could be a role for intraperitoneal IUT prior to 18 weeks of gestation when intravascular IUT using the umbilical vein becomes feasible.

Resolution of hydrops is expected with an adequate IUT regimen. Specific to the diagnosis of ATM is the recommendation to measure Hb Bart in addition to total Hb in the pretransfusion sample. Following the initial gradual correction of anemia, the target hematocrit following transfusion after 24 weeks of gestation should be chosen at the higher end of the recommended range (40% to 50%).[67] An HbA level >10 g/dL and Hb Bart <20% in the pretransfusion fetal blood sample is expected with
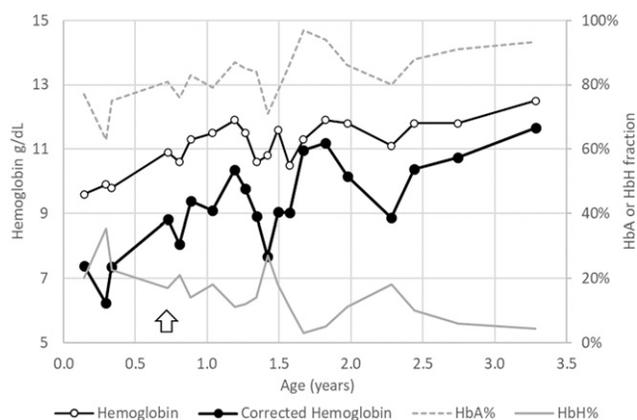
Figure 4. Case 4 depiction. A newborn with α-thalassemia major (—SEA/—SEA) was initially transfused with a goal of maintaining total Hb of 9 to 10 g/dL in the pretransfusion period (case 4). This was associated with effective functional Hb (total Hb − sum of HbH and Hb Bart) between 6 and 7 g/dL. Transfusion regimen was modified (arrow) to maintain HbA 9 to 10 g/dL, which was achieved with total Hb of ~11 g/dL in the pretransfusion blood sample.

these goals. The delivery is planned at 37 to 38 weeks with the last IUT no later than 35 weeks of gestation.[67]

Perinatal events in ATM following suboptimal fetal management are characterized by a high incidence of preterm birth, intrauterine growth retardation, cesarean delivery, birth trauma, and difficult resuscitation. Newborns exhibit respiratory distress, pulmonary hypertension, organomegaly, effusions, and hyperbilirubinemia.[61,62] Some may have congenital anomalies affecting the genitourinary system. Anemia is profound and further distinguished by a high proportion of nonfunctional Hb Bart if >2 to 3 weeks have elapsed from the last intrauterine transfusion. An urgent simple transfusion with 5 to 10 mL/kg of high hematocrit red cell unit is usually given. When the proportion of Hb Bart is very high, an exchange transfusion will rapidly improve tissue oxygenation. The goal during the first few weeks is to maintain total Hb >12 g/dL and Hb Bart <20% while the need for critical care continues.

Following stabilization, infants are in a transition period up to 6 months with intensive transfusion support under close monitoring. This period is marked by the switch from Hb Bart to HbH, resolution of hepatosplenomegaly and cardiomegaly, improvement in thrombocytopenia and transaminitis, and the establishment of consistent weight gain. Transfusions aim to maintain nadir total Hb >12 g/dL with the total nonfunctional Hb (Hb Bart plus HbH) <20%. The interval between transfusions is initially 2 weeks, but gradually lengthened to 3 weeks. Red cell antigens should be determined by genetic testing to provide antigen-matched blood. At the end of 6 months, infants transition to a chronic transfusion protocol in which pretransfusion HbA is >9.0 g/dL and transfusion frequency is 3 to 4 weeks. Following the absolute HbA level instead of total Hb is important, otherwise children with ATM are at risk for undertransfusion.[63] Infusion centers lacking access to rapid Hb electrophoresis or high-performance liquid chromatography can aim to maintain the pretransfusion total Hb at 10.5 to 11 g/dL and reticulocyte count <500 000/µL.[63] Splenectomy is not recommended in the management of ATM. Transfusional iron overload is observed early within a few months after birth, but the assessment and management of iron in ATM is not well defined. Because of the concerns over hepatic inflammation and renal immaturity, chelation is postponed until 12 months of age.

Summary

The management of TDT should be adapted to the heterogeneity conferred by various genotypes. Although a universal transfusion protocol for TDT is unfeasible, common principles underlie the long-term goals of transfusion therapy for individuals with thalassemia. With improvement in life expectancy, decisions about initiation and intensity of transfusion support in TDT should be guided by long-term natural history studies that span the various life stages.

Conflict-of interest disclosure

Off-label drug use

None disclosed.

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