Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline

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BACKGROUND: Red blood cell (RBC) transfusions remain essential in the treatment of patients with sickle cell disease (SCD) and β-thalassemia. Alloimmunization, a well-documented complication of transfusion, increases the risk of delayed hemolytic transfusion reactions, complicates crossmatching and identifying compatible units, and delays provision of transfusions. Guidance is required to optimize the RBC product administered to these patients.

STUDY DESIGN AND METHODS: An international, multidisciplinary team conducted a systematic review and developed, following the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) methodology, recommendations to assist treating physicians and transfusion specialists in their decision to select RBCs for these patients.

RESULTS: Eighteen studies (17 clinical studies and one cost-effectiveness study) were included in the systematic review. The overall quality of the studies was very low. In total, 3696 patients were included: 1680 with β-thalassemia and 2016 with SCD.

CONCLUSION: The panel recommends that ABO D CcEe K–matched RBCs are selected for individuals with SCD and β-thalassemia, even in the absence of alloantibodies, to reduce the risk of alloimmunization. In patients with SCD and β-thalassemia who have developed clinically significant alloantibodies, selection of RBCs antigen negative to the alloantibody is recommended, if feasible. In these patients, selection of more extended phenotype-matched RBCs will likely reduce the risk of further alloimmunization. However, given the limited availability of extended phenotype-matched units, attention should be given to ensure that a delay in transfusion does not adversely affect patient care.

ABBREVIATIONS: DHTR(s) = delayed hemolytic transfusion reaction(s); GRADE = Grading of Recommendations, Assessment, Development, and Evaluation; ICTMG = International Collaboration for Transfusion Medicine Guidelines; SCD = sickle cell disease.

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Funding for the systematic review and guideline development was partially provided by Canadian Blood Services. Canadian Blood

Sickle cell disease (SCD) and β-thalassemia are inherited red blood cell (RBC) disorders. Simple or exchange transfusions remain a life-sustaining therapy in individuals with SCD. RBC transfusion is recommended to prevent complications of SCD including stroke in individuals with abnormal transcranial Doppler ultrasound velocities and those who previously experienced an overt or clinically silent stroke.[1] RBC transfusion is also recommended prophylactically in the perioperative period as well as during the treatment of acute complications, for example, acute chest syndrome.[1] For β-thalassemia, transfusion therapy is a lifelong requirement for survival and is used to suppress ineffective erythropoiesis and improve growth and development in children.[2]

Whereas transfusions are effective in preventing morbidity in patients with SCD and β-thalassemia, alloimmunization is a well-documented risk that is associated with hemolytic transfusion reactions of varying severity, autoantibody formation, and delays in patient care when identification of compatible units becomes a challenge.[3-5]

At present, more than 35 blood group systems have been described. Genetic differences among individuals translate into different amino acid sequences of proteins either expressed at the surface of the RBC membrane or involved in determining the specificity of enzymes, for example, glycosyltransferases. These differences ultimately result in different blood group antigens expressed at the RBC surface. The *RHD* and *RHCE* genes, coding RhD and CE antigens, respectively, are characterized by a high number of genetic alleles leading to the expression of variants. Polymorphisms in other blood group systems are often limited to single amino acid differences. Given the genetic basis of blood group systems, it is not surprising that the frequency of certain antigens and their variants differs among ethnicities and alloimmunization risk is affected by the heterogeneity between donor and patient populations.

No international consensus exists for antigen matching in patients with SCD and β-thalassemia. Some transfusion medicine services provide preventive phenotype (or genotype) extensive matching for C, c, E, e, and K antigens in addition to routine ABO and D. Additional extended matching for Jk^a, Jk^b, Fy^a, Fy^b, and S, s is offered at some centers. In contrast, others provide ABO and D-matched RBCs and switch to more extensive matching only if alloantibodies are detected. The cost of extended matching and the potential delay in providing phenotype-matched products may be prohibitive for some transfusion services.

In addition to phenotype-matched RBCs, fresh RBCs are postulated to reduce the risk of alloimmunization. In mice, leukoreduced RBCs that were 14 days old led to higher alloantibody levels than fresher units.[6] The benefit of providing fresh RBCs has been investigated in other populations but not extensively in patients with hemoglobinopathies.[7]

An international team of adult and pediatric hematologists, hematopathologists, methodologists, and transfusion medicine physicians completed a systematic review and developed recommendations to assist treating physicians and transfusion specialists in their decision of optimizing the RBC product when transfusing individuals with β-thalassemia or SCD. Specifically, the panel addressed whether the extent of RBC antigen matching and/or RBC unit age resulted in a reduction in mortality, transfusion reactions, alloimmunization, or mean RBC units transfused. These recommendations are intended for transfusion medicine physicians as well as any physician intending to transfuse patients with hemoglobinopathies and apply to patients who require chronic or isolated RBC transfusion.

MATERIALS AND METHODS

Information sources and search

The search strategy was developed by two of the authors (NS and ST) with the assistance of library information specialists. The search was applied to the electronic databases MEDLINE, EMBASE, Cochrane Library, and CINAHL from 1946 to September 2016. References identified from bibliographic searches and by panel members were also included. The search strategy and text words are shown in Appendix A.

Study selection

Citations were independently assessed in duplicate to identify studies that met the following inclusion criteria: (1) an original study; (2) included five or more patients with hemoglobinopathies; (3) compared RBC genotyping/phenotyping/antigen matching with unmatched RBCs or focused on the age of RBCs transfused to these patients; (4) included any of the following outcomes—mortality, a reduction in the proportion of patients transfused or the number of units transfused, the frequency of transfusion reactions including alloimmunization or cost effectiveness; and (5) published in English. Case reports and editorials were excluded.

Services as a funding agency did not have any role in the design, analysis, and interpretation of the data or preparation, review, and approval of the manuscript. Funding for development of the podcast was provided by a BloodTechNet award from Canadian Blood Services.

Received for publication October 10, 2017; revision received January 18, 2018; and accepted January 24, 2018.

doi:10.1111/trf.14611

TRANSFUSION 2018;58;1555–1566

TABLE 1. GRADE criteria[12]

Level of evidence	Explanation
Strong	Further research is unlikely to change our confidence in the estimate of effect.
Moderate	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low	Any estimate of effect is very uncertain.

If there was disagreement, the full report was retrieved and independent assessment was repeated. Disagreements for inclusion were resolved by consensus.

Data abstraction

Data were extracted from each of the studies and the quality was assessed in duplicate (Appendix B, Tables S1-S5 [available as supporting information in the online version of this paper]).

Assessing the quality of individual studies

The assessment of the risk of bias of individual primary studies was based on the checklist developed by the Cochrane Collaboration.[8] The assessment of economic analysis was based on the checklist developed by Evers and colleagues.[9]

Method of analysis

A meta-analysis was not conducted due to considerable heterogeneity in the measurement of study outcomes; thus, only a qualitative analysis is provided.

Development of recommendations

The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) tool was used for the development of recommendations. GRADE incorporates the quality of the evidence, benefits and risks, and resource utilization.[10,11] The level of evidence was graded as strong, moderate, weak, or very weak based on the GRADE criteria listed in Table 1.[12] The strength of the recommendation was evaluated as strong or weak based on the level of available evidence. Evidence was downgraded, according to the GRADE criteria, if there was inconsistency, small benefit, absence of high-quality evidence, and imprecise estimates of benefits or harms. A strong recommendation was made based on the GRADE criteria if the panel was "confident that the desirable effects of adherence to a recommendation balanced any undesirable effects of the intervention."[13] A weak recommendation was made if the panel determined that the "desirable effects of adherence to a recommendation likely outweighed any undesirable effects," but the panel was uncertain about the balance of benefits and risks.[13] Weak recommendations were also made to reflect differences in individual patient circumstances that would need to be taken into consideration. The term *should* was used to reflect strong recommendations, and *should probably* was used to reflect weak recommendations. Weak recommendations may not be applicable to all patients. All of the studies were noncontrolled trials; thus, the estimates for net benefit and net harm could not be accurately depicted in the GRADE tables but are described following each recommendation.

An electronic survey was sent to all members of the panel to assess agreement with the recommendations. Disagreements were resolved with group discussions. A recommendation that could not be resolved following discussion was sent for a vote with majority decision (50% or more) leading to the acceptance of the recommendation. Members who had potential conflicts of interest were not excluded from voting. The guideline was sent for review by several societies: AABB, American Society for Hematology, British Society for Haematology, Cooley's Anemia Foundation, UK Forum on Haemoglobin Disorders, Network of Rare Blood Disorder Organizations, Sickle Cell Disease Association of America, Sickle Cell Disease Association of Canada, Thalassemia International Federation, and Thalassemia Society of UK. Societies were not requested to approve the guideline.

This guideline will be updated 3 years following publication.

RESULTS

Study selection

A total of 3482 citations were identified (Fig. 1). Of these, 2924 were screened after duplicates were removed and 24 full-text articles were assessed to be eligible. Six of the 24 full-text articles were excluded (Fig. 1). There were two additional studies identified that focused on the age of RBCs.[14,15]

Characteristics of the studies

Our systematic review included 18 studies (Appendix B, Tables S1-S5). Fourteen were full-text reports of clinical studies (four prospective,[16,17] eight retrospective,[13,18-24] and two could not be determined[25,26]), three were abstracts of retrospective clinical studies,[27-29] and one was a cost-effectiveness study.[30] Of the 18 clinical studies, 14 were single-center,[15,17,19-26,28,29] three were multicenter,[13,14,18] and one did not report center status.[27] Six studies included patients with β-thalassemia syndromes[15-17,23,25,28] and 12 with SCD.[13,14,18-22,24,26,27,29]

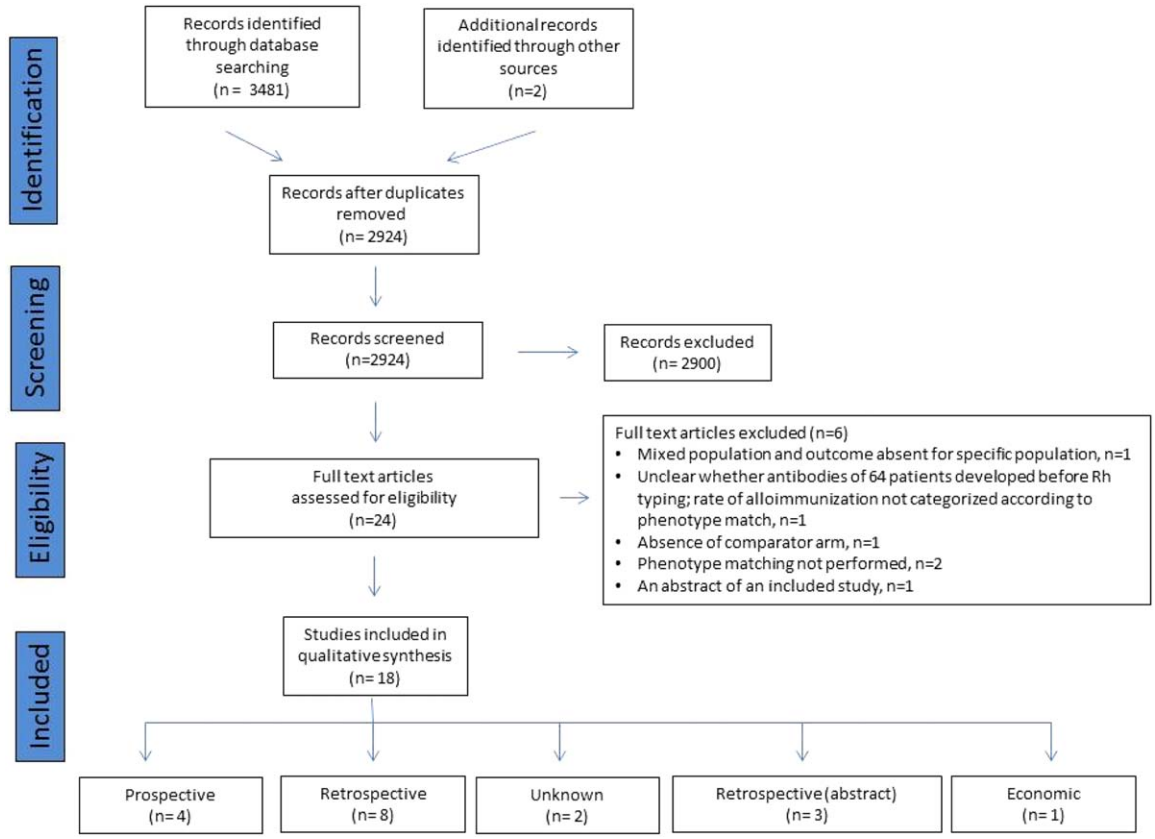

Fig. 1. Flow diagram of included studies. [Color figure can be viewed at wileyonlinelibrary.com]

Tables S1, S2, and S4 describe the characteristics of the studies included.

Outcomes of the studies

Intervention arms and clinical outcome

Of the 3489 patients included in the 18 studies (Appendix B), 1680 (45%) had β-thalassemia syndromes and 2016 (55%) had SCD. Sample sizes ranged from 23 to 1200 patients. Table S1 describes the different intervention arms as well as the sample size for each study. Two percent (five of 233) of patients with SCD[21] died of hyperhemolysis and 3% (two of 64) of patients with β-thalassemia[23] died of iron overload–related complications. Febrile nonhemolytic,[13,22] allergic,[13,22,24] hemolytic,[13,23] and delayed hemolytic transfusion reactions (DHTRs)[19,22] were reported infrequently. Transfusion reactions and the frequency of allo- and autoimmunization are displayed in detail in Table S1.

Economic study outcomes

One economic analysis was identified (Appendix B, Tables S4 and S5)[30] which simulated prospective CEK or CcEe K Fy^a Fy^b Jk^a Jk^b S s versus history-based antigen matching to compare cost and alloimmunization prevention. Implementing prospective limited matching instead of history-based limited matching for CEK was estimated to cost an additional US$766 million over 10 years and results in 2072 fewer alloimmunization events (Table S5). Implementing prospective extensive matching for CcEe K Fy^a Fy^b Jk^a Jk^b S s was estimated to cost an additional $1.86 billion and results in 2424 fewer alloimmunization events compared to history based-matching for CEK over a 10-year interval. Using prospective matching for a transfusion naive cohort will cost $369,482 to $769,284/single alloimmunization event prevented. Using prospective matching instead of history-based limited matching will cost $252,816/single alloimmunization event prevented over 10 years for individuals who may have received a transfusion. Cost saving of history-based limited matching over prospective limited matching is maintained if the expense of matching was more than $20. Not all costs were considered, however (e.g., finding a unit for an alloimmunized patient).

Quality of the studies

The risk of bias assessment for the clinical studies is shown in Fig. 2. Serious and critical risks of bias in the 16 studies occurred in the domains of confounding (10 of 16), selecting participants (eight of 16) and measurement of intervention (seven of 16). Moderate risks were

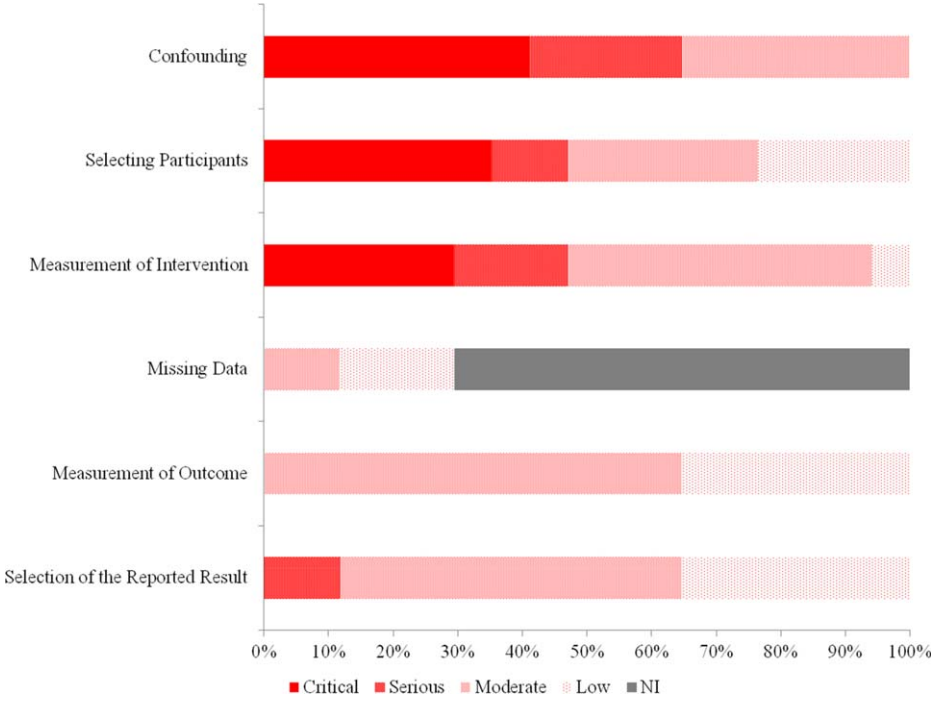

Fig. 2. Risk of bias. NI = no information [Color figure can be viewed at wileyonlinelibrary.com]

identified in the domains of measurement of outcomes (11 of 16) and selection of reported results (nine of 16). Sixty-nine percent (11 of 16) of the studies did not report missing data.

The risk of bias assessment for the studies that analyzed the age of RBCs is demonstrated in Table S3. The studies were both assessed to have a high risk of bias for the measurement of the association with the age of blood as patients were not consistently administered units with either long or short durations of storage. The mean age of blood was used to correlate clinical outcomes and age of blood.

The checklist for the assessment of the quality of the economic study is illustrated in Table 2. The quality was limited by the lack of using a systematic review as a basis for the analysis, lack of inclusion of potential delay of transfusion, and the limited description of outcomes.

Recommendations

The GRADE evidence profile (Table 3) indicates the low quality of evidence supporting the recommendations. Table 4 and Fig. 3 provide a summary of the recommendations including implications for centers in low-resource settings. Recommendations 1 and 4 required several iterations to ensure that the majority of panel members agreed with the recommendations.

Recommendation 1: Patients with SCD who do not have alloantibodies and who are anticipated to have a transfusion (simple or exchange transfusion) should probably be transfused with CcEe and K-matched RBCs to reduce the risk of alloimmunization (low quality of evidence, weak recommendation).

Three retrospective studies[18,21,22] observed a lower risk for alloimmunization in patients with SCD transfused with ABO and D along with CcEe K,[18,21] or CEK[22] compared to standard matched RBCs; ABO D. Sakhalkar and colleagues[22] described a reduced frequency of autoimmunization and did not observe transfusion reactions in the limited CEK-matched group, whereas febrile, allergic, and DHTRs were noted with the ABO D-matched group. A prospective multicenter study confirmed the feasibility of limited CEK matching and suggested lower rates of alloimmunization and hemolytic transfusion reactions.[13] Reduced frequency of alloimmunization[24,27] and autoimmunization[26] in patients with SCD was also observed in studies[24,26,27] investigating the effect of more extended phenotyping. Mortality and the proportion transfused RBCs were not addressed in any study.

In a retrospective study, Chou and colleagues[19] evaluated the effect of CEK phenotype matching with RBCs from African American donors and observed that 45% of the chronically and 12% of the episodically transfused patients with SCD unexpectedly formed alloantibodies against D, C, E, or e. High-resolution *RH* genotyping revealed significant genetic diversity in the Rh system that was not detected with serological phenotyping. Altered RH alleles were present in 87% of patients with SCD, and some Rh antibodies were explained by inheritance of altered RH. Overall, 20 of 50 (40%) Rh antibodies in

TABLE 2. Quality of the economic study (according to Evers and colleagues[9])

Items		Kacker et al. 2014[30]
1.	Is the study population clearly described?	Yes
2.	Are competing alternatives clearly described?	Yes
3.	Is a well-defined research question posed in answerable form?	Yes
4.	Is the economic study design appropriate to the stated objective?	No
5.	Is the chosen time horizon appropriate to include relevant costs and consequences?	Yes
6.	Is the actual perspective chosen appropriate?	Yes
7.	Are all important and relevant costs for each alternative identified?	No
8.	Are all costs measured appropriately in physical units?	Yes
9.	Are costs valued appropriately?	No
10.	Are all important and relevant outcomes for each alternative identified?	Yes
11.	Are all outcomes measured appropriately?	No
12.	Are outcomes valued appropriately?	No
13.	Is an incremental analysis of costs and outcomes of alternatives performed?	Yes
14.	Are all future costs and outcomes discounted appropriately?	Yes
15.	Are all important variables, whose values are uncertain, appropriately subjected to sensitivity analysis?	Yes, proportion transfused
16.	Do the conclusions follow from the data reported?	Yes
17.	Does the study discuss the generalizability of the results to other settings and patient/client groups?	Yes
18.	Does the article indicate that there is potential conflict of interest of study researcher(s) and funder(s)?	Yes but no disclosure
19.	Are ethical and distributional issues discussed appropriately?	No

individuals with the corresponding antigen and eight of 29 (28%) in individuals without the corresponding antigen receiving antigen-negative blood were associated with a DHTR.

Providing matched RBCs is recommended, although patients may not have developed alloantibodies in the past, as there is a potential for alloantibody development with future transfusion. RBCs matched for CcEe and K can be provided by phenotyping or genotyping RBCs. The use of phenotyping or genotyping will depend on the costs of each method in each jurisdiction. Genotyping appears to be more accurate. When unexpected Rh antibodies are detected despite the serologic presence of the antigen or provision of Rh-matched RBCs, molecular investigation (i.e., Rh genotyping) may be warranted.

Recommendation 2: Patients with SCD who have one or more clinically significant alloantibodies should be transfused with antigen-negative blood to the corresponding antigen(s) alloantibody(ies), if feasible (low quality of evidence, strong recommendation).

Once alloantigens are recognized by the receptors of a patient's T lymphocytes, B lymphocytes are stimulated, proliferate, and become antibody-producing plasma cells as well as memory cells. In the absence of the provoking antigen, alloantibodies will gradually disappear from the circulation. Upon renewed exposure to the alloantigen, memory cells will rapidly produce alloantibodies, which can result, in the case of clinically significant alloantibodies, in a DHTR. In patients with SCD, this can be associated with bystander hemolysis.[4] Antigen-negative RBCs should therefore be selected for individuals with SCD that have developed clinically significant alloantibodies, even when the alloantibodies are no longer detectable in the patient's plasma.[31]

Some patients develop multiple clinically significant alloantibodies. In the emergency setting, RBCs negative for all corresponding antigens may not be available and the clinical condition of the patient may require an at-risk transfusion. The term *feasible* in the recommendation applies to scenarios where the well-being of a patient may preclude extended antigen matching when RBCs are needed urgently. For alloantibodies against low-incidence antigens or those that are not typically associated with clinical significance, crossmatching may replace the selection of antigen-negative RBCs, regardless of their screening test results in the emergency setting.[31]

Consideration should be given to inform individuals of their alloantibodies, for example, by providing them with cards/letters that can be presented at each hospitalization to ensure that they receive antigen-negative RBCs.

Recommendation 3: Patients with SCD who have one or more alloantibodies should probably be transfused with CcEe K Fy^a Fy^b Jk^a Jk^b S s–matched RBCs to reduce the risk of alloimmunization, if feasible and if matching does not cause undue delays that adversely affect patient care (low quality of evidence, weak recommendation).

The development of an alloantibody is dependent on several factors including the RBC product and donor characteristics. Nonetheless, it has been previously demonstrated[32,33] and is accepted that some individuals who develop one alloantibody have the propensity to develop additional antibodies. Three studies investigated the effect of extended phenotyping beyond limited CEK on alloimmunization. Tahhan and colleagues[24] did not observe alloimmunization in the CEK, Fy^a, Fy^b, S–matched group versus 34.8% alloimmunization in patients receiving a combination of phenotype-matched and unmatched transfusions (9% of patients were previously

TABLE 3. GRADE profile of RBC specifications for hemoglobinopathies

Number of studies	Study design	Quality assessment: Study limitation	Inconsistency	Indirectness	Imprecision	Impact	Quality	Importance
Alloimmunization (sickle cell disease)								
4	Nonrandomized	Very serious*	Serious†	Not serious	Serious‡	Two studies showed reduced alloimmunization rates with CcEe K 35 of 236 (15%) matching compared to ABO D 193 of 497 = (39%) in patients with sickle cell disease.[21,22] One study found similar alloimmunization rates with ABO D matching (24 of 85 = 28%) and CcEe K Jk^a Jk^b Fy^a Fy^b ± MNSs P1 Le^a Le^b matching (3 of 12 = 25%) but individuals receiving matched RBCs had received unmatched RBCs prior to matching.[26]	Very low	Important
Autoimmunization (sickle cell disease)								
2	Nonrandomized	Very serious*	Very serious†	Not serious	Very serious‡	One study found reduced autoimmunization with extended matching 1 of 113 (1%) in CcEe K versus 39 of 387 (10%) in ABO D.[22] One study found 11 of 85 (13%) autoimmunizations with ABO D matched blood and 5 of 12 (42%) with extended matching for Cc Ee K Jk^a Jk^b Fy^a Fy^b ± MNSs P1 Le^a Le^b blood, but individuals receiving matched RBCs had received unmatched RBCs prior to matching.[26]	Very low	Important
Alloimmunization (thalassemia syndrome)								
4	Nonrandomized	Very serious*	Not serious	Not serious	Serious‡	Two studies found a 19% (28 of 147) alloimmunization rate in the ABO D matched group compared to 6% (14 of 218) in the CcEe K matched group.[17,25] One study found 8 of 211 (4%) in the ABO D-matched group, 0/46 (0%) in the CcEe K–matched group and 8 of 227 (3%) in the ABO D shifted or started on CEK.[15] One study found 18 of 55 (33%) in the ABO D group and 1 of 35 (3%) in the ABO D shifted to or started on CEK.[23]	Very low	Important

* The selection of patients, the lack of consistency of testing and follow-up were limitations. There was failure to adequately control for confounding and incomplete follow-up.
† The outcomes were inconsistent.
‡ The sample size was not predetermined to power the study.

TABLE 4. Recommendations for RBC transfusions in patients with hemoglobinopathy*

1	Patients with SCD who do not have alloantibodies and who are anticipated to have a transfusion (simple or exchange transfusion) should probably be transfused with CcEe and K-matched RBCs to reduce the risk of alloimmunization (low quality of evidence, weak recommendation). RBCs matched for CcEe and K can be provided by phenotyping or genotyping RBCs. The use of phenotyping or genotyping will depend on the costs of each method in each jurisdiction. Genotyping appears to be more accurate. Providing matched RBCs is recommended, although patients may not have developed alloantibodies in the past, as there is a potential for alloantibody development with future transfusion. Phenotyping or genotyping are provided by several centers prior to the first transfusion.
2	Patients with SCD who have one or more clinically significant alloantibodies should be transfused with antigen negative blood to alloantibody(ies), if feasible (low quality of evidence, strong recommendation). Consideration should be given to inform individuals of their alloantibodies by for example providing them with cards/letters that can be presented at each hospitalization to ensure that they receive antigen-negative RBCs.
3	Patients with SCD who have one or more alloantibodies should probably be transfused with CcEe K Fy^a Fy^b Jk^a Jk^b S s matched RBCs to reduce the risk of alloimmunization, if feasible and if matching does not cause undue delays that adversely affect patient care (low quality of evidence, weak recommendation).
4	Patients with thalassemia syndromes who do not have alloantibodies and who require RBC transfusion should probably be transfused with CcEe and K-matched RBCs to reduce the risk of alloimmunization (low quality of evidence, weak recommendation). RBCs matched for CcEe and K can be provided by phenotyping or genotyping RBCs. The use of phenotyping or genotyping will depend on the costs of each method in each jurisdiction. Genotyping appears to be more accurate. Providing matched RBCs is recommended although patients may not have developed alloantibodies in the past, as there is a potential for alloantibody development with future transfusion. Phenotyping or genotyping are provided by several centers prior to the first transfusion.
5	Patients with thalassemia syndromes who have one or more clinically significant alloantibodies should be transfused with antigen negative blood to the alloantibody(ies), if feasible (low quality of evidence, strong recommendation). Consideration should be given to inform individuals of their alloantibodies by, for example, providing them with cards/letters that can be presented at each hospitalization to ensure that they receive antigen-negative RBCs.
6	Patients with thalassemia syndromes who have one or more alloantibodies should probably be transfused with CcEe K Fy^a Fy^b Jk^a Jk^b S s matched RBCs to reduce the risk of alloimmunization, if feasible and if matching does not cause undue delays that adversely affect patient care (low quality of evidence, weak recommendation).

* The recommendations are in addition to standard ABO matching.

alloimmunized). Boateng and colleagues[27] observed a 50% reduction in alloimmunization prevalence with CEK matched for patients without previous alloantibodies and CEK Fy^a Fy^b Jk^a Jk^b–matched RBCs for patients with previous alloantibodies, compared to a transfusion regimen combining both phenotype-matched and unmatched RBC transfusion. Ambruso and colleagues[26] observed lower autoimmunization in patients transfused with an extended matching protocol that included Rh, Kell, Kidd, and Duffy antigens. Even with extended phenotyping, 25% of the patients developed new alloantibodies, possibly due to errors during antigen matching. None of the studies investigated whether specific patient characteristics contributed to the development of the alloantibodies.

The supply of antigen-negative RBCs becomes more challenging when more extended matching is applied.[34] Especially in case of emergencies, communication between the transfusion medicine specialist and the attending physician are essential to define the most appropriate approach for an individual patient. Previous reports have detailed the immunogenicity of antigens, which can be used as a guide to match for specific antibodies if extended matching is not available.[35-37]

As discussed above, patients presenting with alloantibodies against the Rh system, despite being identified as having the antigen serologically, may warrant further molecular investigation.

Recommendation 4: Patients with thalassemia syndromes who do not have alloantibodies and who require RBC transfusion should probably be transfused with CEK-matched RBCs to reduce the risk of alloimmunization (low quality of evidence, weak recommendation).

Similar to SCD, mortality and the proportion of all patients transfused were not identified in any study. In a pilot study, Spanos and colleagues[25] observed significantly lower alloimmunization rates in β-thalassemia patients receiving limited CcEe K–matched RBCs compared to patients receiving ABO and D-matched cells. A reduction in alloimmunization rate after CEK matching was also observed in a retrospective study,[23] although differences in frequency of leukoreduction of the transfused RBCs among groups was a potential confounding factor. Two prospective studies investigating the effect of limited cEK or CcEe versus ABO and D matching[16,17] only partially confirmed previous findings.[25] Pujani and colleagues[16] did not observe alloimmunization events in patients with β-thalassemia major receiving cEK-matched leukoreduced RBCs compared to a low alloimmunization rate in the ABO and D-matched group. Michail-Merianou and colleagues[17] noted a higher alloimmunization prevalence in the ABO and D-matched group compared to the limited CcEe K–matched group, but presumably due to low sample size, this difference was not statistically significant.

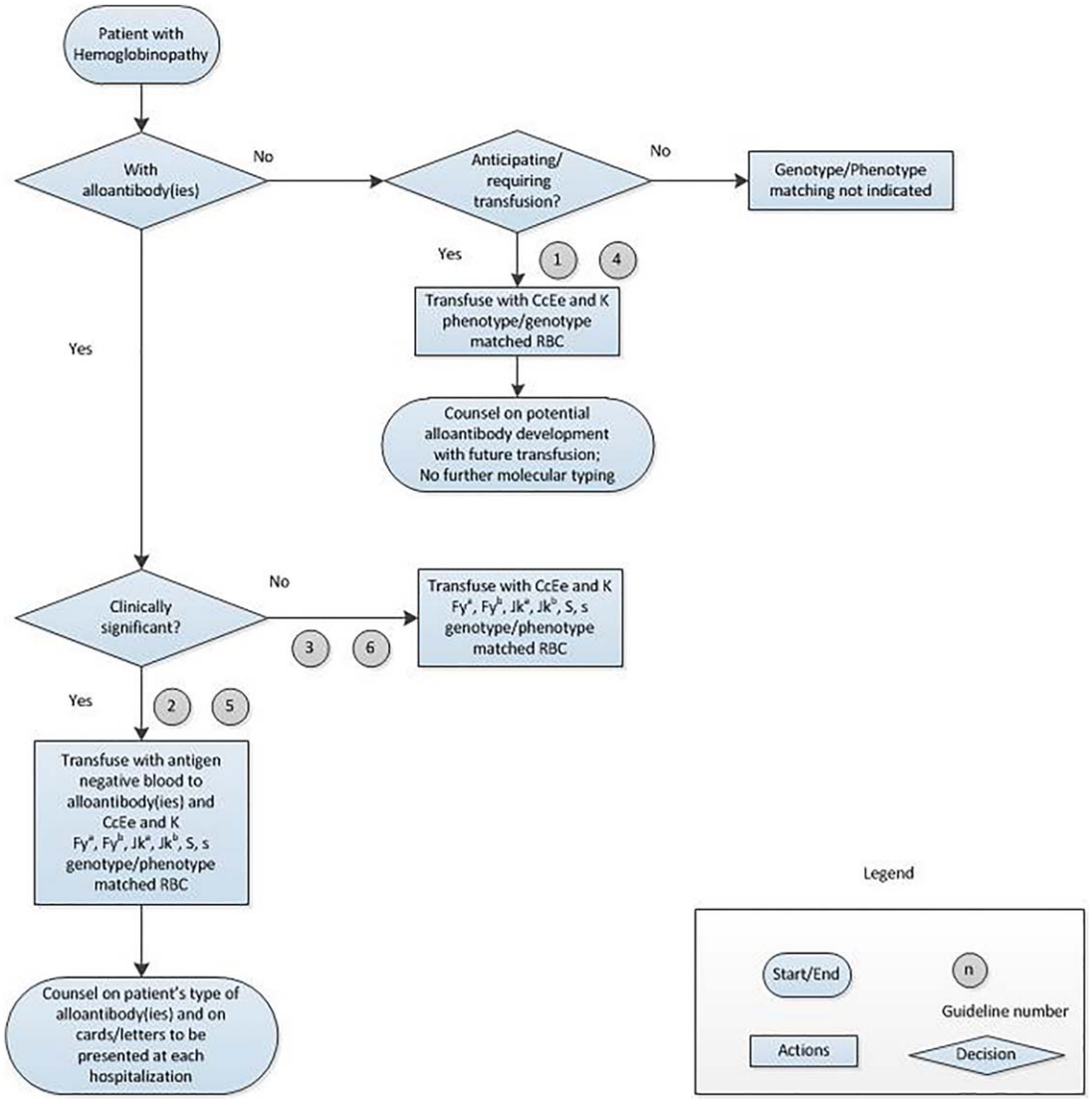

Fig. 3. Algorithm for RBC transfusion in patients with sickle cell disease and thalassemia syndromes. [Color figure can be viewed at wileyonlinelibrary.com]

Recommendation 5: Patients with thalassemia syndromes who have one or more clinically significant alloantibodies should be transfused with antigen-negative blood to the corresponding antigen(s), if feasible (low quality of evidence, strong recommendation).

As discussed above, renewed exposure to the alloantigen results in a rapid production of antibodies by memory cells that can subsequently provoke DHTR. Antigen-negative blood should therefore be selected for individuals with β-thalassemia who have developed clinically significant alloantibodies even when the alloantibodies are no longer detectable in the patient's plasma. As described earlier, in case of clinically not significant alloantibodies as well as in case of alloantibodies against low-frequency antigens, the selection of antigen-negative units may be replaced by crossmatching[31,38] in case of emergencies; thus, a transfusion that may be associated with increased risk of a transfusion reaction may be required. Similar to patients with SCD, cards/letters that can be presented at each hospitalization to ensure that they receive antigen-negative RBCs should be provided to these patients.

Recommendation 6: Patients with thalassemia syndromes who have one or more alloantibodies should probably be transfused with CcEe K Fy^a Fy^b Jk^a Jk^b S s–matched RBCs to reduce the risk of alloimmunization, if feasible and if matching does not cause undue delays that adversely affect patient care (low quality of evidence, weak recommendation).

Several studies suggest a reduction in alloimmunization risk in individuals with β-thalassemia when limited CEK matching for RBCs is applied. No studies have

investigated phenotype matching of RBCs beyond CcEe K in patients with β-thalassemia. Extrapolating from data in patients with SCD that demonstrate lower rates of alloimmunization with more extended antigen matching, a reduction in alloimmunization risk is expected in patients with β-thalassemia transfused with extended antigen-matched RBCs.

A recommendation was not developed for the duration of RBC storage as only two studies focused on the duration of RBC storage: one in patients with SCD[14] and one in patients with thalassemia[15] (Tables S2 and S3). In 165 patients with SCD, longer duration of RBC storage was found to be associated with the development of alloimmunization, but once patients who had received frozen RBCs were excluded from the analysis, the hazard ratio of alloimmunization associated with longer duration of storage was no longer statistically significant.[14] The duration of storage did not affect the hemoglobin concentration of 31 patients with thalassemia major, but the report did not discuss the effect on the number of RBC units transfused, the frequency of alloimmunization, transfusion reactions, or other morbidities.[15]

The above recommendations were predominantly based on studies performed in the United States, Europe, and other high- or high-middle-income countries. In addition, heterogeneity between donors and recipients, particularly in patients with SCD, in these countries, increases the risk for RBC alloimmunization. The majority of patients with SCD, however, live in sub-Saharan Africa.[39,40] A recent meta-analysis of RBC alloimmunization in (mainly chronically) transfused patients in sub-Saharan Africa[41] showed that even in this setting where donors and recipients are racially similar, RBC alloimmunization occurs in approximately 7% of patients. In these countries with very limited health care resources, pretransfusion testing is often limited to assuring ABO compatibility. As a first step to improving transfusion safety for patients in low-resource settings, the only recommendation that may be feasible and cost-effective to implement would be to perform antibody screens/identification in chronically transfused patients and provide RBCs that do not have the corresponding antigen(s) to those who have developed a clinically significant RBC alloantibody. If this is impossible, then at a minimum, RBCs that have been crossmatched and found compatible with a technique capable of detecting clinically significant RBC alloantibodies should be provided. Informing patients of their RBC alloantibodies, however, is essential universally.

DISCUSSION

An international panel of experts in RBC transfusion completed a systematic review of the literature and developed recommendations to assist physicians and transfusion specialists in their decision to provide extended matched RBCs or RBCs with shorter storage duration to individuals with β-thalassemia or SCD. Although a patient representative was not included in the panel, the potential that alloimmunization could affect the quality of life of the involved patients was taken into consideration and recommendations were sent to the Cooley's Anemia Foundation, a patient group, for review. A podcast and slide deck are available at ICTMG.org to assist clinicians with disseminating the guideline.

The quality of the selected studies was very low and limits the strength of the formulated recommendations. Limited description of patient characteristics and the RBC product limited the development of recommendations according to these features, as did the small sample sizes of patients with dissimilar SCD or thalassemia genotypes. A randomized clinical trial would ideally provide high-quality evidence to demonstrate that prophylactic CEK-matched RBCs do reduce alloimmunization, although this practice is already the standard of care in some institutions. The potential impact of alloimmunization was recently highlighted by a case series of patients with SCD with fatal outcomes.[4] DHTRs with or without hyperhemolysis, transfusion delays due to fear from previous transfusion reactions, or unavailability of compatible RBCs all contributed to patient mortality. Targeted donor recruitment to obtain a more diverse donor pool will be needed to improve the availability of matched RBCs. Future studies will be needed to establish the role of genotype-matched RBCs in ethnically diverse patient populations. In the meantime, discussion between frontline clinicians and transfusion medicine specialists regarding transfusion urgency and the potential for finding compatible blood is paramount.

ACKNOWLEDGMENTS

International Collaboration for Transfusion Medicine Guidelines (ICTMG) Members. **Shubha Allard**, MD, FRCP, FRCPath, Barts Health NHS Trust and NHS Blood & Transplant; **Celso Bianco**, MD, formerly with America's Blood Centres; **Jeannie Callum**, BA, MD, FRCPC, CTBS, University of Toronto, Canada; **Veerle Compernolle**, MD, PhD, Belgian Red Cross-Flanders, Belgium; **Dean Fergusson**, MHA, PhD, University of Ottawa, Canada; **Mark Fung**, MD, PhD, Fletcher-Allen Health Care, USA; **Andreas Greinacher**, MD, University of Greifswald, Germany; **Heather Hume**, MD, FRCPC, Université de Montréal, Canada; **Lani Lieberman**, MD, University of Toronto, Canada; **Michael Murphy**, MD, FRCP, FRCPath, FFPath, University of Oxford, UK; **Susan Nahirniak**, MD, FRCPC, University of Alberta, Canada; **Katerina Pavenski**, MD, FRCPC, University of Toronto, Canada; **Joanne Pink**, MBBS, FRACP, FRCPA, Australian Red Cross Blood Services, Australia; **Arjuna Ponnampalam**, MD, FRCPC, University of Manitoba, Canada; **Paolo Rebulla**, Ospedale Maggiore Policlinico, Italy; **Nadine Shehata**, MD, FRCPC, MSc, University of Toronto, Canada; **Cynthia So-Osman**, Groene Hart Ziekenhuis,

Netherlands; **Simon J. Stanworth**, MA, MRCP, DPhil, FRCPath, University of Oxford, UK; **Zbigniew M. Szczepiorkowski**, MD, PhD, Dartmouth-Hitchcock Medical Centre, USA; **Susano Tanael**, MD MMA, Canadian Blood Services, Canada; **Alan T. Tinmouth**, MD, FRCPC, MSc, University of Ottawa, Canada; **Erica Wood**, MBBS, FRACP, FRCPA, Monash University, Australia. The authors thank Dr Matthew Yan for the development of the podcast, Rouhi Fazelzad for the search strategy, and Sylvia Torrance and Kimberly Figures for administrative assistance.

CONFLICT OF INTEREST

SC received funding from Doris Duke Charitable Foundation for research on expanding genomic approaches to transfusion therapy for patients with SCD. GD received honoraria from Grifols S.A. and published a commentary on future of RBC alloimmunization risk reduction. JH provides consultancy to Novartis and Pfizer. CDJ provides consultancy and received research funding from Immucor. WS provides consultancy at Momenta Pharmaceuticals and was a panel member in NHLBI Evidence-based Sickle Cell Disease Guidelines. NS is a consultant for Canadian Blood Services. The remaining authors have disclosed no conflicts of interest. Disclosures were requested yearly from the guideline panel but members were not excluded from voting on recommendations.

REFERENCES

1. Yawn BP, Buchanan GR, Afenyi-Annan AN, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. JAMA 2014;312:1033-48.
2. Goss C, Giardina P, Degtyaryova D, et al. Red blood cell transfusions for thalassemia: results of a survey assessing current practice and proposal of evidence-based guidelines. Transfusion 2014;54:1773-81.
3. Noizat-Pirenne F, Tournamille C. Relevance of RH variants in transfusion of sickle cell patients. Transfus Clin Biol 2011;18:527-35.
4. Nickel RS, Hendrickson JE, Fasano RM, et al. Impact of red blood cell alloimmunization on sickle cell disease mortality: a case series. Transfusion 2016;56:107-14.
5. Chou ST, Liem RI, Thompson AA. Challenges of alloimmunization in patients with haemoglobinopathies. Br J Haematol 2012;159:394-404.
6. Hendrickson JE, Hod EA, Spitalnik SL, et al. Storage of murine red blood cells enhances alloantibody responses to an erythroid-specific model antigen. Transfusion 2010;50:642-8.
7. Heddle NM, Cook RJ, Arnold DM, et al. Effect of short-term vs. long-term blood storage on mortality after transfusion. N Engl J Med 2016;375:1937-45.
8. Sterne JAC HJ, Reeves BC; Development Group for ACROBAT-NRSI. A Cochrane Risk of Bias Assessment Tool: for Non-Randomized STudies of Interventions (ACROBAT-NRSI), Version 1.0.0 [cited 2017 Sep]. Available from http://www.riskofbias.info. Accessed September, 2017.
9. Evers S, Goossens M, de Vet H, et al. Criteria list for assessment of methodological quality of economic evaluations: consensus on health economic criteria. Int J Technol Assess Health Care 2005;21:240-5.
10. Cochrane IMS. RevMan 5 [cited 2016 Apr]. Available from http://tech.cochrane.org/revman/gradepro.
11. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction–GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 2011;64:383-94.
12. Atkins D, Best D, Briss PA, et al. Grading quality of evidence and strength of recommendations. BMJ 2004;328:1490
13. Schunemann HJ, Oxman AD, Akl EA, et al. Moving from evidence to developing recommendations in guidelines. Proc Am Thorac Soc 2012;9:292-92.
14. Vichinsky EP, Luban NL, Wright E; Stroke Prevention Trial in Sickle Cell Anemia, et al. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. Transfusion 2001;41:1086-92.
15. Desai PC, Deal AM, Pfaff ER, et al. Alloimmunization is associated with older age of transfused red blood cells in sickle cell disease. Am J Hematol 2015;90:691-5.
16. Pasricha SR, Frazer DM, Bowden DK, et al. Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with beta-thalassemia major: a longitudinal study. Blood 2013;122:124-33.
17. Pujani M, Pahuja S, Dhingra B, et al. Alloimmunisation in thalassaemics: a comparison between recipients of usual matched and partial better matched blood. An evaluation at a tertiary care centre in India. Blood Transfus 2014;12 Suppl 1:s100-4.
18. Michail-Merianou V, Pamphili-Panousopoulou L, Piperi-Lowes L, et al. Alloimmunization to red cell antigens in thalassemia: comparative study of usual versus better-match transfusion programmes. Vox Sang 1987;52:95-8.
19. Sins JW, Biemond BJ, van den Bersselaar SM, et al. Early occurrence of red blood cell alloimmunization in patients with sickle cell disease. Am J Hematol 2016;91:763-9.
20. Chou ST, Jackson T, Vege S, et al. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. Blood 2013;122:1062-71.
21. Mijovic A, Perera IG, Thein SL. Red blood cell alloimmunization in sickle cell disease-prevalence and trends: a single-center cross-sectional study from United Kingdom. Transfusion 2013;53:3279-80.
22. Ameen R, Al Shemmari S, Al-Bashir A. Red blood cell alloimmunization among sickle cell Kuwaiti Arab patients who received red blood cell transfusion. Transfusion 2009;49:1649-54.
23. Sakhalkar VS, Roberts K, Hawthorne LM, et al. Allosensitization in patients receiving multiple blood transfusions. Ann N Y Acad Sci 2005;1054:495-9.
24. Singer ST, Wu V, Mignacca R, et al. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. Blood 2000;96:3369-73.

25. Tahhan HR, Holbrook CT, Braddy LR, et al. Antigen-matched donor blood in the transfusion management of patients with sickle cell disease. Transfusion 1994;34:562-9.
26. Spanos T, Karageorga M, Ladis V, et al. Red cell alloantibodies in patients with thalassemia. Vox Sang 1990;58:50-5.
27. Ambruso DR, Githens JH, Alcorn R, et al. Experience with donors matched for minor blood group antigens in patients with sickle cell anemia who are receiving chronic transfusion therapy. Transfusion 1987;27:94-8.
28. Boateng LA, Andrew C, Schonewille H. Alloimmunization in transfused sickle cell patients: effect of RBC antigen matching. Int J Lab Hematol 2014;36:118.
29. Al Riyami AZ, Al-Mahrooqi SA, Al Hinai S, et al. Transfusion therapy in thalassemia intermedia: ten years of experience at a tertiary care university hospital. Transfusion 2013;53: 185A-6A.
30. Castilho L, Riberiro K, Guelsin G, Gilli S, et al. J. Molecular matching of red blood cells is superior to the serological matching in sickle cell disease patients. Vox Sanguinis 2012; 103:219.
31. Kacker S, Ness PM, Savage WJ, et al. Cost-effectiveness of prospective red blood cell antigen matching to prevent alloimmunization among sickle cell patients. Transfusion 2014;54:86-97.
32. Milkins C, Berryman J, Cantwell C, et al. Guidelines for pretransfusion compatibility procedures in blood transfusion laboratories. British Committee for Standards in Haematology. Transfus Med 2013;23:3-35.
33. Higgins JM, Sloan SR. Stochastic modeling of human RBC alloimmunization: evidence for a distinct population of immunologic responders. Blood 2008;112:2546-53.
34. Higgins JM, Sloan SR. Other factors may contribute to differences between sickle cell and non-sickle cell patients. Blood 2010;115:4315-6.
35. Castro O, Sandler SG, Houston-Yu P, et al. Predicting the effect of transfusing only phenotype-matched RBCs to patients with sickle cell disease: theoretical and practical implications. Transfusion 2002;42:684-90.
36. Evers D, Middelburg RA, de Haas M, et al. Red-blood-cell alloimmunisation in relation to antigens' exposure and their immunogenicity: a cohort study. Lancet Haematol 2016;3: e284-92.
37. Daniels G. The clinical significance of blood group alloantibodies and the supply of blood for transfusion [cited 2017 Feb 23]. Available from http://hospital.blood.co.uk/media/27446/spn2143-the-clinical-significance-of-blood-group-alloantibodies-and-the-supply-of-blood-for-transfusion.pdf.
38. Williams WJ, Beutler E, Erslev AJ. Hematology. 4th ed. New York: McGraw-Hill; 1990.
39. Cappellini MD, Cohen, A., Porter, J., et al. Guidelines for the management of transfusion dependent thalassaemia (TDT). Nicosia, Cyprus: TIF Publications; 2014.
40. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010;115:4331-6.
41. Piel FB. The present and future global burden of the inherited disorders of hemoglobin. Hematol Oncol Clin North Am 2016;30:327-41.
42. Ngoma AM, Mutombo PB, Ikeda K, et al. Red blood cell alloimmunization in transfused patients in sub-Saharan Africa: a systematic review and meta-analysis. Transfus Apher Sci 2016;54:296-302.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Characteristics and outcome of the studies
Table S2. Characteristics of the studies assessing age of RBCs
Table S3. Risk of bias of studies assessing age of RBCs
Table S4. Characteristics of the economic study
Table S5. Outcome of the economic study