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The erythrocyte alloimmunisation in patients with sickle cell anaemia: a systematic review

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SUMMARY

Transfusion therapy is a common practice in the treatment of anaemia and can cause erythrocyte alloimmunisation. To systematise data related to erythrocyte alloimmunisation in patients with sickle cell disease (SCD), a bibliographic search was carried out in September 2017 to search for studies in four electronic databases. (i) Referring to the original work, (ii) being cohort or case–control, (iii) having been developed with individuals with SCD and (iv) having evaluated the erythrocyte alloimmunisation. Two reviewers identified the articles for inclusion in the study, extracted the predetermined data and carried out the evaluation of the methodological quality of the work. 21 studies were selected; the studies included data on 20 636 individuals (children and adults), were mostly published in the last 10 years, were developed in the United States and had high methodological quality. The occurrence of erythrocyte alloimmunisation ranged from 4·4 to 76%, and there was a higher rate of alloimmunisation against antigens of the Rh system. The risk factors for alloimmunisation were age; gender (female); red blood cell (RBC) units received; presence of ≥1 autoantibodies, TNF-α, interleukin (IL1B), human leukocyte antigens (HLA)-DRB1 gene polymorphisms; first blood transfusion (BT) after 5 years of age, transfusion episodic, multiple or during inflammatory events, acute chest syndrome (ACS) and vase-occlusive crisis (VOC); increased percentage of CD41 T memory cells; and positive direct antiglobulin test. Transfusion policies should be developed to protect the patient and his or her health based on the main factors associated with its incidence.

Key words: BT, multi-transfused, red blood cell alloimmunisation, sickle cell.

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Sickle cell disease (SCD) is one of the most common hereditary pathologies of genetic character in the world (Fernandes, 2017; Kassim & Sharma, 2017). SCD is associated with clinical manifestations such as pain and organ failure, depending on nutritional status, age and comorbidities of the individual (Zago & Pinto, 2007; Al-Mousawi *et al.*, 2015; Meier *et al.*, 2017). Transfusion therapy with packed red cells is used in the treatment of this disease (Davis *et al.*, 2017; Nevitt *et al.*, 2017). Approximately half of these patients receive multiple transfusions throughout their lives, and approximately 7·5% begin a system of chronic transfusion (Zheng & Maitta, 2016). However, this therapy can be associated with an increased risk of hyperhemolysis, transient aplasia and erythrocyte alloimmunisation (Miller *et al.*, 2013; Kelly *et al.*, 2016; Makarovska-Bojadzieva *et al.*, 2017).

Erythrocyte alloimmunisation is the result of an immune response of the recipient, initiated through the recognition of erythrocyte antigens, proteins and membrane glycoproteins with the formation of antibodies and memory cells. Its occurrence is variable and is related to age, gender, number of transfusions received and phenotypic differences between recipients and blood donors (BDs) (Helman *et al.*, 2011; Neurosci, 2016). Erythrocyte alloimmunisation limits the availability of packed red cells compatible for future transfusions in addition to being a risk factor for severe hyperhemolysis. Therefore, it is necessary to manage the stock of packed red cells with negative antigens for antibodies of a higher frequency in SCD (Natukunda *et al.*, 2010).

Knowing that transfusion therapy is a common practice in the treatment and prevention of complications of SCD and that it can have consequences such as alloimmunisation, this systematic review of the literature (SRL) aims to analyse the results of studies that assessed the incidence of erythrocyte alloimmunisation and the most common types of alloantibodies detected and risk factors in individuals with SCD. This study aimed to contribute more complete scientific data for the improvement of transfusion therapy, decreasing the occurrence of this outcome and contributing to the longevity of sickle cell patients.

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METHODS

Literature search and study selection

This study was an SRL performed according to the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses Group Guidelines* (Moher *et al.*, 2009).

In the month of September 2017, a single researcher (E.G.C.G) carried out a search for articles published since 1997 using online available abstracts in the electronic databases Cochrane Systematic Reviews (CDSR), Latin American and Caribbean Literature in Health Sciences (LILACS) Capes Journals, US National Library of Medicine, National Institutes of Health (Pubmed) and Scopus using the following key words: (Sickle Cell) and (Isoantibodies or RBC Alloimmunization) and (Multitransfused or BT or Erythrocyte Transfusion). No restrictions were made regarding the language. Another strategy was a manual search of lists of references of the articles.

Then, two authors read the titles and abstracts of the studies (E.G.C.G. and L.C.O.) independently. The inclusion criteria required that the articles should (i) be original, (ii) be cohort or case–control, (iii) be developed with individuals with SCD and (iv) evaluate erythrocyte alloimmunisation. The studies that appeared to meet these criteria were selected for a full-text reading. Disagreements among the reviewers were resolved by consensus and, when necessary, by consulting a third author (J.F.A.N.).

Data extraction

Using proper form, an author (E.G.C.G.) carried out the data extraction, including the following information: (i) place of implementation, (ii) age of participants, (iii) study design, (iv) sample size, (v) objectives, (vi) blood data, (vii) transfusion protocol, (viii) alloantibodies found, (ix) evaluated variables and (x) results. Another author (L.A.F.M.) reviewed this activity.

Quality assessment

The methodological quality of the studies was assessed independently by two authors (E.G.C.G. and L.C.O.) using the Newcastle–Ottawa Scale (NOS) (Wells *et al.*, 2013). In this scale, a star can be assigned for each item related to the selection of participants, comparability and results of the studies. The maximum number of stars is 9, with a score of 7–9 stars considered high quality, 4–6, moderate quality, and < 3, low methodological quality.

The kappa coefficient (k) was used to evaluate the concordance among the authors. The initial agreement between the two authors regarding the total number of stars given to the work was considered substantial ($k = 0{\cdot}82$). Discordant cases were discussed until consensus was reached or the opinion of the third author (J.F.N.N.) was consulted.

RESULTS

Literature search and characteristics of the included studies

The selection process enabled the identification of 37 articles in PubMed and Scopus, 25 in PubMed and 4 in Lilacs. In addition, examination of the reference lists of articles did not identify any other additional studies. After the articles were read, 21 were selected (Fig. 1) (Al-Saeed, 1997; Padmanabhan & Chandrasekaran, 2000; Olujohungbe *et al.*, 2001; Bashawri, 2007; Meunier *et al.*, 2008; Natukunda *et al.*, 2010; Zanette *et al.*, 2010; Helman *et al.*, 2011; Aly *et al.*, 2012; Wahl *et al.*, 2012; Diarra *et al.*, 2013; O'Suoji *et al.*, 2013; Fasano *et al.*, 2014; Desai *et al.*, 2015; Karafin *et al.*, 2015; Michot *et al.*, 2015; Nickel *et al.*, 2015; Sins *et al.*, 2016; Tatari-Calderone *et al.*, 2016; Allali *et al.*, 2017; Sippert *et al.*, 2017).

Most of the studies assessed the frequency of alloimmunisation and were published in the last 10 years, with a variable sample size of 42 (Aly *et al.*, 2012) to 6120 patients (Karafin *et al.*, 2015). The studies included data from 20 636 individuals (children and adults) from different countries and originated in the United States ($n = 8$), France ($n = 4$); Brazil ($n = 3$); Saudi Arabia ($n = 2$); and The Netherlands, Egypt, Uganda, Jamaica and UK (all with $n = 1$ studies) (Table 1).

Occurrence of erythrocyte alloimmunisation

All authors have reported the application of a transfusion protocol (Table 1); however, this did not extinguish the occurrence of alloimmunisation among patients with SCD. The overall mean incidence of erythrocyte alloimmunisation was 28·39%, with a variation of 4·4% (Miller *et al.*, 2013) to 76·0% (Olujohungbe *et al.*, 2001) (Table 2).

As seen by analysing the average general incidence per country, there were higher frequencies reported in the studies developed in UK (76·0%), followed by the United States (34·7%), Brazil (29·8%), France (24·0%), Saudi Arabia (23·9%), Egypt (21·4%), The Netherlands (18·0%) and Uganda (6·1%), with less expression in Jamaica (2·6%) (Table 2).

Of the selected studies, 15 showed a higher rate of alloimmunisation against antigens of the Rh system, showing that they are among the most immunogenic. The second highest rate was found for alloantibody antigens of the Kell system ($n = 3$ articles), followed by the MNS system ($n = 2$) and the Lewis system ($n = 1$) (Table 2).

Risk and protection factors associated with the incidence of erythrocyte alloimmunisation

Age, gender and history of BT were the covariables most frequently assessed in the studies. The main risk factors for alloimmunisation were as follows: age; gender (female); RBC units received; presence of ≥1 autoantibodies, TNF-α, interleukin (IL1B) and human leukocyte antigens (HLA)-DRB1 gene polymorphisms; first BT after 5 years old, transfusion

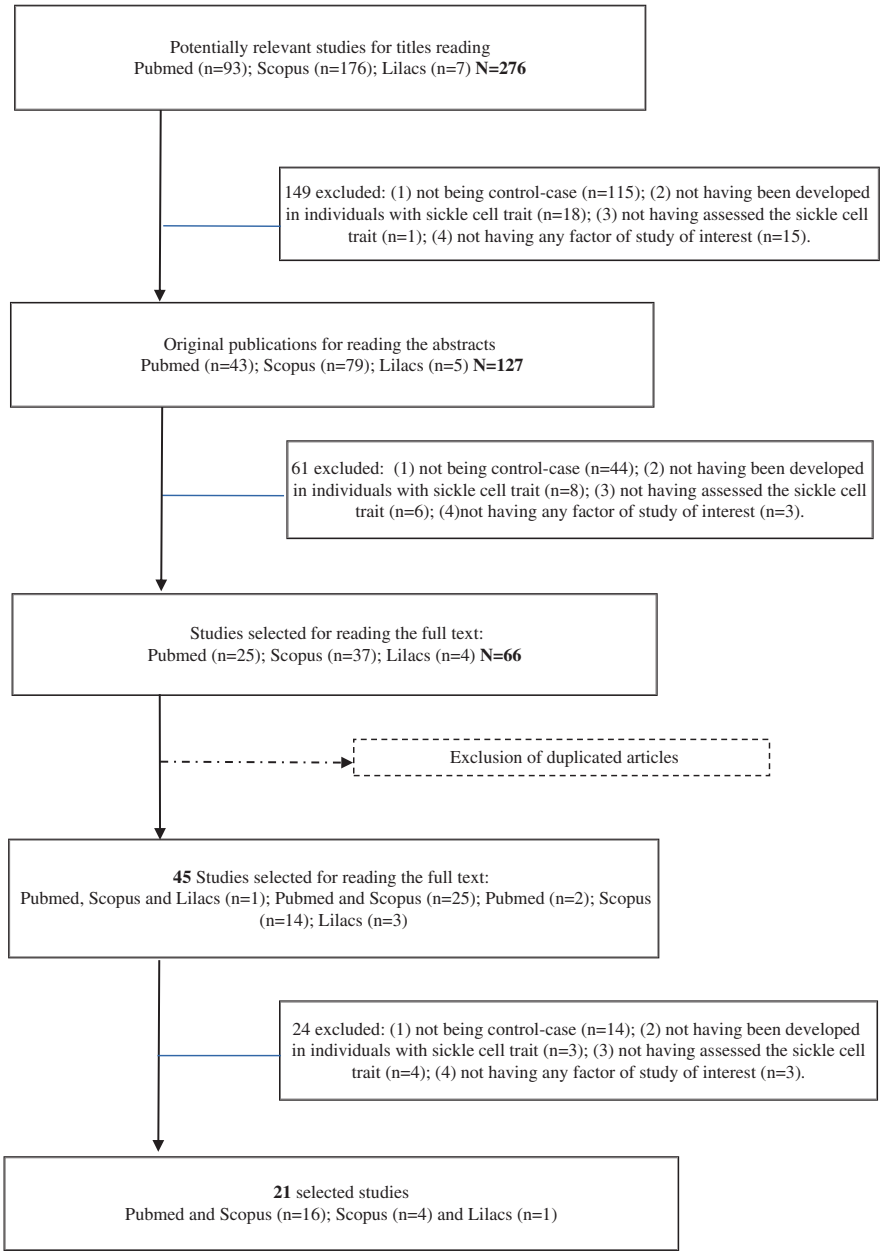

Fig. 1. Flowchart of the selection. Note: *n*, number of observations. Designed in accordance with PRISMA (Moher *et al.*, 2009).

episodic, multiple or during inflammatory events, acute chest syndrome (ACS) and VOC; increased percentage of CD41 T memory cells; and positive direct antiglobulin test. Increased antigen matching (Fasano *et al.*, 2014) demonstrated a protective effect, and HLA-DQ2 ($P = 0{\cdot}02$), -DQ3 ($P = 0{\cdot}02$) and -DQ5 ($P = 0{\cdot}01$) alleles were higher in non-alloimmunised patients (Tatari-Calderone *et al.*, 2016) (Table 2).

Quality assessment

The majority of studies were considered to be of high methodological quality, among which only two received the maximum score, one developed in Brazil (Sippert *et al.*, 2017) and one in The Netherlands (Sins *et al.*, 2016) (Table 2).

DISCUSSION

The present study has three main results. First, the study confirms that the incidence in erythrocyte alloimmunisation is quite variable among the studies. Second, there was evidence that the antibodies against Rh antigens were more frequent. Finally, we verified that this result might be associated with several risk factors and protection, some modifiable and others not, inherent to patients with SCD, the BD and the transfusion process.

Table 1. Description of studies regarding the authors, national origin, age of the patients, study design, sample size, objectives, blood data and transfusion protocol

ID	Author	Origin	Age (years)	Design	*N*	Objectives	Blood data	Transfusion protocol
1	Allali *et al.*, 2017	France	11·9 (7·6–15·4)	Cohort	175	Study the prevalence and risk factors of alloimmunisation.	IAT. 15-cell panel +15-cell enzyme-treated RBC panel (papain and/or trypsin). Serological testing, RBC phenotyping and genotyping.	All patients received leucocyte-reduced, non-irradiated RBC that were phenotypically matched for Rh matching.
2	Sippert *et al.*, 2017	Brazil	Control: 33·0 (18·0–63·0)[2] Alloimmunised: 36·0 (4·0–68·0)[2] Non-alloimmunised: 34·0 (2·0–70·0)	Case–control	449	Investigate the association of alloimmunisation with polymorphisms of HLA and cytokines genes.	UN	The institutional policy for RBC transfusions is to antigen match prospectively at least for Rh matching.
3	Sins *et al.*, 2016	The Netherlands	20·0 (16·0–28·0)	Cohort	245	Elucidate the association between the BT, alloimmunisation and risk factors.	UN	Before 2004, RBC transfusions were only matched for ABO and RhD. In January 2004, extended RBC phenotyping for Rh matching.
4	Tatari-Calderone *et al.*, 2016	USA	16·4 (5·2)[1]	Case–control	204	Analyse the influence of HLA-DRB1 and DQB1 genetic on alloimmunisation.	UN	All patients received leuko-depleted RBC. Prior to 2010, received cross-matched compatible ABO and RhD-matched RBCs. Since 2010, received rhesus -C-, –E- and -K-matched RBCs.
5	Desai *et al.*, 2015	USA	22·6 (12·0–39·0)	Cohort	166	Explore the association of clinical complications and age of RBC with alloimmunisation.	UN	All patients were transfused with RBC units that were, at a minimum, matched for Rh matching.
6	Karafin *et al.*, 2015	USA	BD: 31·8 (14·9)[1] SCD patients: 32·4 (12·2)[1]	Case–control	6120	Evaluated the RBC antigen characteristics of population with SCD and the BD.	Real-time PCR assays for donor genotyping. Solid-phase method (Immucor, Norcross, GA) + polyethylene glycol enhanced or low-ionic-strength saline tube techniques.	It is blood bank policy that all RBC units provided to patients with SCD are antigen-matched for Rh matching by the blood provider, when the serological phenotype is known.
7	Michot *et al.*, 2015	France	23·0 (14·0)[1]	Case–control	188	Evaluate the incidence of haemolytic reactions and alloimmunisation.	Hb electrophoresis. Others clinical data collected from database.	All SCD patients underwent rhesus and Kell RBC phenotyping and a systematic cross-matching test for detecting any antibodies.
8	Nickel *et al.*, 2015	USA	Alloimmunised: 13·0 (5·7–22·1)[2] Non-alloimmunised: 10·7 (2·1–18·5)[2]	Case–control	90	Investigate characteristics of patients who develop RBC alloantibodies.	Tube testing for RBC antibody detection with polyethylene glycol enhancement (Immucor, Norcross, GA, USA) and solid phase (Immucor, Norcross, GA, USA). Serological testing, RBC phenotyping and genotyping.	All patients received RBC units that were Hb S-negative, pre-storage leukocyte-reduced and non-irradiated. These units were phenotype antigen matched for Rh matching.

Table 1. Continued

ID	Author	Origin	Age (years)	Design	*N*	Objectives	Blood data	Transfusion protocol
9	Fasano *et al.*, 2014	USA	UN	Cohort	52	Determine the influence of pro-inflammatory SCD complications at time of BT on alloimmunisation.	Gel methodology (Ortho IDMTS TM IgG Gel cards; Ortho Clinical Diagnostics ID-Micro Typing SystemTM, Rochester, NY, USA).	All units were sickle-negative, pre-storage leuco-reduced. The RBC-matching protocol from 1999 to July 2012 entailed matching for ABO/RhD for all SCD patients until an RBC alloantibody was formed, at which time C, E and K antigen (CEK) matching was initiated. In July 2012, CNMC began CEK primary matching for all patients with SCD.
10	Diarra *et al.*, 2013	France	21·0 (1·0–62·0)[2]	Cohort	133	Determine the prevalence of HIV, HBV, HCV infections and alloimmunisation.	Plate technique. Gel filtration technique using the DiaClonRh-Subgroups + K cards (BIO-RAD, France). Coombs indirect gel filtration technique (CoombsAnti-IgG, BIO-RAD, France).	Patients received 1 and 2 units of phenotyped RBC concentrate compatible with ABO + RhDCEce + K systems on the same day.
11	O'Suoji *et al.*, 2013	USA	Alloimmunised 0–5 (19·2%) 6–10 (11·5%) 11–15 (34·6%) 16–20 (30·8%) 21–25 (3·8%)	Case–control	180	Assess alloimmunisation.	Gel testing (ID-Micro Typing System, Ortho Clinical Diagnostics, Rochester, NY). Phenotyping of donor is performed by tube technique with licensed antisera according to manufacturer's directions (Ortho Clinical Diagnostics).	Yes, but it was not detailed.
12	Aly *et al.*, 2012	Egypt	Alloimmunised: 12·0 (2·4)[1] Non-alloimmunised: 6·2 (2·3)[1]	Case–control	42	Explore the frequency of alloantibodies.	ID Card micro-typing system (Geltear, Switzerland). Dia cell I, II and III were used to screen for the presence of red cell alloantibodies and the Dia panel to identify these alloantibodies.	All patients received fully matched blood for only ABO and Rh (D) antigens.
13	Wahl *et al.*, 2012	USA	ECP: 8·4 (4·4)[1] Conventional transfusion: 17·3 (5·3)[1]	Case–control	45	Compare alloimmunisation rates between patients receiving simple transfusion or ECP.	UN	Yes, but it was not detailed.
14	Helman *et al.*, 2011	Brazil	25·0 (16·0–66·0)[2]	Cohort	57	Identify the incidence of alloimmunisation.	Column agglutination and gel centrifugation (ID-Diapanel and ID-Diapanel-P, DiaMed AG). Elution of antibodies + phenotyping.	Yes, but it was not detailed.

Table 1. Continued

ID	Author	Origin	Age (years)	Design	*N*	Objectives	Blood data	Transfusion protocol
15	Natukunda *et al.*, 2010	Uganda	12·0 (2·0–44·0)[2]	Case–control	428	Determine the frequency of alloimmunisation.	Standard three-cell panel. IAT, a low-ionic-strength saline–enhanced gel centrifugation technique (DiaMed ID, Micro Typing System, DiaMed, Cressier sur Morat, Switzerland) with poly-specific anti-human globulin (rabbit anti-immunoglobulin [Ig]G and monoclonal anti-C3d). Panels of reagent RBCs of selected phenotypes. DNA was extracted from buffy coat samples (using the QIAamp DNA blood mini kit, Qiagen, Hilden, Germany) using an RHD multiplex PCR.	Patients received packed RBC transfusions compatible with their ABO and D phenotypes and that were not leuko-reduced.
16	Zanette *et al.*, 2010	Brazil	Alloimmunised: 30·1 (8·1)[1] Non-alloimmunised: 34·3 (12·4)[1]	Case–control	108	Compare the clinical profile of transfused SCD patients with and without alloantibodies.	UN	Before the year 2004, the RBC for transfusion were matched only with standard ABH-D. From 2004, a new transfusion policy for SCD patients was applied including screening for Rh matching.
17	Meunier *et al.*, 2008	France	21·0 (1·0–62·0)[2]	Cohort	206	Show that, to BT of SCD patients, it is necessary to take into account their immunohematological characteristics.	RAI and the direct compatibility test, performed in an IAT.	ABO/RH/KEL grouping and extended phenotype (FY/JK/MNS) are performed from the first transfusion.
18	Bashawri, 2007	Saudi Arabia	28·8 (15·0)[1]	Cohort	350	Assessed the frequency of alloimmunisation.	Two-cell panel with homozygous expression of the antigens (Ortho-Clinical Diagnostics, USA. and DiaMed, ID-Micro typing system, Morat, Switzerland). Commercial RBC panel with known antigens (Resolve Panel A and B, Ortho Clinical Diagnostics, USA) and DiaMed IDDIA panel (without enzymes) and IDDia Panel P (with enzymes) against the patient's serum. DAT. Cross match.	All patients received fully matched blood for only ABO and D antigens.

Table 1. Continued

ID	Author	Origin	Age (years)	Design	*N*	Objectives	Blood data	Transfusion protocol
19	Olujohungbe *et al.*, 2001	Jamaica and UK	Jamaican patients: 19·7 (17·0 – 22·0)[2] UK patients: 26·2 (14·0 – 31·6)[2]	Cohort	227	Compare the transfusion history and frequency of red cell antibodies.	Microtyping gel cards (DiaMed-ID, DiaMed GB, Midlothian, UK) and a low-ionic-strength IAT (Diacell I and II Screening Cells, DiaMed). 11-cell panel.	African origin, transfusion polices are more conservative and it is considered the ethnic phenotyping protocol for Rh; procedure not adopted in the UK patients.
20	Padmanabhan & Chandrasekaran, 2000	USA	19·0 – 57·0	Cohort	71	Find the incidence and clinical significance of alloantibodies.	UN	Patients received compatible BT with no attempt to match for red cell antigens unless patient developed an antibody.
21	Al-Saeed, 1997	Saudi Arabia	UN	Cohort	111	Detect alloimmunisation.	UN	Yes, but it was not detailed.

DAT, direct antiglobulin test; Hb, haemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; IAT, rescindir antiglobulin test; *N*, size sample; PCR, polymerase chain reaction; RAI, irregular agglutinins; UN, uninformed; USA, United States of America; CNMC, Children's National Medical Centre; HLA-, human leukocyte antigen; TNF-, tumor necrosis factor-alpha.

[1]Mean (SD).

[2]Median (IQR).

The variation in the incidence of erythrocyte alloimmunisation found can be justified by different reasons. The studies showing the lowest rates included individuals with lower age, which shows the difference in the risk of alloimmunisation in older patients (Moreira Júnior *et al.*, 1996; AABB, 1999). In addition, different methods of antibody identification were used in the analyses, among which there is a variable sensitivity of detection. Centrifugation in tube and gel can increase diagnostic sensitivity in relation to other tube-only centrifugation methodologies (e.g. Murao & Viana, 2005). In addition, a lower occurrence of erythrocyte alloimmunisation might reflect greater phenotype compatibility between BDs and patients (AABB, 1999). Some studies were also limited by their retrospective design, lack of data related to blood analysis and obtaining information from third-party databases, compromising the quality of the information and possibly representing a bias.

Based on the study of Olujohungbe *et al.* (2001), it has been verified that the ethnic correlation between donors and recipients reflects a difference in the frequency of alloimmunisation between two countries (Jamaica = 2·6%; UK = 76%). In Jamaica, both donors and recipients are predominantly of African origin, and transfusion polices are more conservative and consider an ethnic phenotyping protocol for Rh, which is a procedure not adopted in the United Kingdom. In addition, Jamaican patients were younger and therefore had a lower risk of alloimmunisation {Median 19·7 years [interquartile range (IQR): 17·0 – 22·0] vs the median of 26·2 years (IQR: 14·0 – 31·6)].

Rh antibodies were more frequent, followed by alloantibodies antigens of the Kell, MNS and the Lewis systems, consistent with the scientific literature (Moreira Júnior *et al.*, 1996). The intensity of the immunogenicity varied in accordance with the polymorphism of each system of blood group (BG) antigens, where holders of a lipoprotein structure, such as those of the Rh system, Kell, Kidd and Duffy, are more prone to alloimmunisation (Girello & Kuhn, 2011).

It is necessary to administer the stock of packed red cells with antibody-negative antigens at a higher frequency in the sickle cell population (Natukunda *et al.*, 2010). The respect of the Rh/Kell phenotype suggests a significant likelihood of transfusion in intra-ethnic situations, which implies a higher probability of phenocompatibility in other BG systems.

Alloimunisation depends on factors such as immune responses, frequency of transfusions, immunogenicity of the antigen, age and the receiver's gender (Murao & Viana, 2005; Bashawri, 2007; Santos *et al.*, 2007). The studies by Fasano *et al.* (2014), Sins *et al.* (2016) and Allali *et al.* (2017) associated the increase in the incidence of alloimmunisation with sporadic blood transfusions. In addition, Fasano *et al.* (2014) and Allali *et al.* (2017) found a higher risk of sensitisation in individuals who received BTs in the presence of previous inflammation. Studies show that an inflammatory condition in receptors favours the development of alloimmunisation in mice (Smith *et al.*, 2012) and in humans (Papay *et al.*, 2012; Fasano *et al.*, 2014). Desai *et al.* (2015) relates the validity of the hemocomponents, generating the accumulation of oxidative factors during

Table 2. Description of studies regarding alloantibodies detected, co-variables analysed, main results and methodological quality

ID	Alloantibodies detected	Co-variables	Main results	Quality[1]
1	Anti-M ($n=15$); Anti-Kp(a) ($n=5$); Anti-Le(a) ($n=4$); Anti-Le(b) ($n=3$); Anti-S ($n=3$); Anti-D ($n=2$); Others ($n=8$).	Age; Gender; GO; Hb; History of BT; Haemolytic reaction; VOC; ACS; Splenectomy; Osteomyelitis; Stroke.	The prevalence of alloimmunisation was 7·4%, being bigger for episodically transfused patients. Main risk factors for alloimmunisation were increased with regard to RBCU received and the presence ≥1 autoantibodies.	High (8 pts)
2	Anti-E (38·8%); Anti-C (32·8%); Anti-K (20·9%); Anti-D (16·4%); Anti-S (13·4%); Anti-Di(a) (13·4%); Anti-e (9·0%); Anti-Jk(b) (7·5%); Others (30·0%).	Age; Gender; History of BT; Use of Hydroxyurea; Splenectomy.	The prevalence of alloimmunisation was 14·9%. Patients with the TNFA, IL1B and HLA-DRB1 gene polymorphisms were at a higher alloimmunisation risk.	High (9 pts)
3	Before the study: Anti-M ($n=5$); Anti-Le(a) ($n=4$); Anti-I ($n=2$); Others ($n=2$). During the study: Anti-E (33·0%); Anti-C (30·0%).	Age; DO; Gender; Genotype; History of pregnancy; History of BT.	Alloimmunisation occurred in 18·0% patients. Receiving the first BT after 5 years old was an independent risk factor (HR = 2·3; 95% CI: 1·0 – 5·1). Incidental BT and exposure to matched units confer a higher alloimmunisation risk.	High (9 pts)
4	Anti-E (54·2%); Anti-C (51·8%); Anti-K (50·6%); Anti-S (18·0%); Anti-V (10·8%); Anti-Fya (10·8%); Anti-M (8·4%); Anti-Jsa (7·2%); Others (20·4%).	Age; Gender; Type of HLA; RBCU.	There were 41·7% alloantibody-positive. Analysis of HLA-DQ showed that HLA-DQ2 ($P=0·02$), -DQ3 ($P=0·02$) and -DQ5 ($P=0·01$) alleles were higher in non-alloimmunised patients.	High (8 pts)
5	Anti-C; Anti-Cob; Anti-Cw; Anti-E; Anti-Fya; Anti-Jka; Anti-Jkb; Anti-Jsa; Anti-K; Anti-Lea; Anti-M; Anti-S; Anti-V[2]	Avascular necrosis; ACS; Gender; Genotype; Indication for BT; Leg ulcers; Retinopathy; Stroke; TRV; Use of Hydroxyurea.	19 patients developed new alloantibodies. Antibody was significantly associated with the age of RBCU (HR = 3·5; 95% CI: 1·71 – 7·11), for an RBCU that was 7 days old and (HR = 9·8; 95% CI: 2·6 – 35·9) for a unit that was 35 days old, 28 days after BT.	High (7 pts)
6	Before the study: Anti-D (1·9%); Anti-C (3·7%); Anti-E (1·9%); Anti-K (7·4%); Anti-S (3·7%); Others (7·6%). During the study: Anti-D (3·7%); Anti-C(3·7%); Anti-e (1·9%); Anti-JS (3·7%); Others (7·6%).	Age; Gender.	12 patients (22·2%) had alloantibodies, and five of these developed these antibodies while receiving Rh and K antigen-matched.	High (8 pts)

Table 2. Continued

ID	Alloantibodies detected		Co-variables	Main results	Quality[1]
7	ECP group: Anti-C (7·0%); Anti-Kpa (4·0%); Anti-Lea (3·0%); Anti-E (4·0%); Anti-Cw (2·0%); Others (9·0%).	ST group: Anti-C (3·0%); Anti-M(7·0%); Anti-Kpa (2·0%); Anti- Lea (2·0%); Anti-S (4·0%); Anti-Fy5 (2·0%); Anti-Jkb (3·0%); Others (15·0%).	Age; BG; ECP; Gender; G6PD-deficient; Hb phenotype; Haemolytic reaction; Autoimmune disease. n of ST; Parvovirus B19; RBCU.	The prevalence of alloimmunisation was 33% in the ECP and 22·0% in the ST group ($P = 0·179$). The alloimmunisation/RBCU ratio was lower in the ECP group ($P < 0·001$).	High (8 pts)
8	Before the study: Anti-E ($n = 2$); Anti-K ($n = 2$); Anti-C(w) ($n = 1$); Anti-Js(a) ($n = 2$); Anti-Fy(a) ($n = 1$); Anti-Fy(b) ($n = 1$); Anti-M ($n = 1$).	During the study: Anti-C ($n = 3$); Anti-E ($n = 4$); Anti-K ($n = 3$); Anti-Go(a) ($n = 4$); Anti-Kp(a) ($n = 5$); Anti-Js(a) ($n = 3$); Anti-S ($n = 3$); Others ($n = 13$).	Age; Gender; History of BT; Stroke; RBCU; Splenectomy; Subset % of ICC	RBC alloimmunisation was present in 29·0% patients and were significantly older, had more RBCU exposures and had a significantly increased percentage of CD41 T memory cells.	High (7 pts)
9	Anti-E (30·4%); Anti-C (25·3%); Anti-K (16·5%); Anti-Fy(a) (8·9%); Anti-V (5·1%); Anti-S (3·8%); Anti-Jkb (2·5%); Anti-M (2·5%); Anti-Jk(a) (1·3%); Others (3·8%).		Age of RBCU; Inflammatory event; Level of matching; Storage solution.	52 patients received 3166 BT, of which 128 resulted in alloantibodies. BT during inflammatory events, ACS and VOC showed associations with alloimmunisation. Increased antigen matching demonstrated a protective effect.	High (7 pts)
10	Anti-C (2·2%); Anti-D (1·1%); Anti-c (1·1%).		Age; Gender; HIV; HBV; HCV; History of BT; Type of Hb.	The alloimmunisation was observed in 4·4% of patients.	High (7 pts)
11	Anti-C (18·7%); Anti-E (14·6%); Anti-Kidd (12·5%); Anti-S (10·4%); Anti-Kell (10·4%); Anti-D (6·2%); Anti-e (4·2%); Anti-Duffy (4·2%); Others (18·8%).		Age; Gender; History of BT; Sickle cell genotype.	26 patients (14·4%) developed new antibodies. The majority of alloimmunised patients received episodic BT.	High (7 pts)
12	Anti-K (7·1%); Anti-E (4·8%); Anti-C (4·8%).		Age; BG; Gender; History of BT.	21·4% patients developed alloantibodies. There was association between alloantibody and the rate of BT.	High (7 pts)
13	ECP group: Anti-rhi ($n = 1$).	ST group: Anti-Lea ($n = 1$);Anti-M ($n = 1$); Anti-D ($n = 1$); Anti-C ($n = 1$); Anti-Kpa ($n = 1$).	Age; Gender; History of BT; RBCU.	The alloimmunisation rate was 0·055 per 100 U. The rate of antibody formation was 0·040 per 100 U in the ECP and 0·171 in the ST group ($P = 0·04$). The alloantibodies formed per 100 U was 0·013 in the ECP and 0·143 in the ST group ($P = 0·03$).	High (7 pts)
14	Anti-K (26·6%); Anti-C (20·0%); Anti-Diego (20·0%); Anti-E (6·6%); Anti-Fy^a (6·6%); Anti-Jkb (6·6%); Anti-D (6·6%); Anti-LutA (6·6%).		Age; BG; Education; History of BT; Rh phenotype; Skin colour.	Irregular antibodies were found in 22·6% patients. The risk of alloimmunisation was higher in who received >3 RBCU in 2 years.	Moderate (6 pts)

Table 2. Continued

ID	Alloantibodies detected		Co-variables	Main results	Quality[1]
15	Anti-E ($n = 10$); Anti-D ($n = 7$); Anti-S ($n = 4$); Anti-C ($n = 2$); Anti-Jka ($n = 2$); Anti-Panreactive ($n = 2$); Others ($n = 5$).		Age; BG; Gender; History of BT; History of pregnancy; RBCU; Usion; 'Febrile illness'.	26 patients (6·1%) possessed RBCU alloantibodies, and 21 (80·7%) of them had received >10 BT.	High (7 pts)
16	Anti-E (39·3%); Anti-K (21·4%); Anti-C (16·1%); Anti-FY(a) (5·3%); Anti-Le(a) (5·3%); Anti-Le(b) (5·3%); Anti-e (3·6%); Anti-D (3·6%); Anti-M (3·6%); Anti-c (3·6%); Others (9·0%).		Age; Cardiac disease; Creatinine; DAT; Fetal hb; Gender; Hb; LDH; Leg ulcer; Neutrophil; Platelet; RBCU; Renal disease; Reticulocyte; Retinopathy; Stroke; Urea; Uric acid; WBC.	Alloimmunisation developed in 52·0% patients. Age, gender and positive DAT show statistically significant differences between groups.	High (7 pts)
17	Rh system (anti-E, anti-C, anti-D and anti-e) (87·5%) Kell and Lutheran system (12·5%).		Age; BG; Gender; Hb genotype; RBCU.	47·0% of the patients had a history of alloimmunisation, whereas only 15·0% produced an antibody in the study.	High (7 pts)
18	Anti-E (42·0%); Nonspecific (38·0%); Anti-c (21·0%); Anti-K (21·0%); Anti-C (6·0%); Anti-S (4·0%); Anti-Fy(a) (4·0%); Anti-Jk(a) (4·0%); %); Others (16·0%).		Age; BG; DAT; Gender; History of BT.	48 patients had developed alloantibodies (13·7%). Some patients had 1 alloantibody, while others ≥1 antibodies.	Moderate (6 pts)
19	Jamaica: Anti-E ($n = 1$); Anti-Kp(a) ($n = 1$); Anti-Le(a) ($n = 2$); Anti-Le(b) ($n = 1$); Anti-S ($n = 1$); Anti-Pan··agglutinin ($n = 1$);	UK: Anti-C ($n = 5$); Anti-D ($n = 3$); Anti-E ($n = 5$); Anti-Fy(a) ($n = 4$); Anti-K ($n = 5$); Anti-Le(a) ($n = 5$); Anti-Le(b) ($n = 4$); Anti-S ($n = 3$); Anti-M ($n = 5$); Others ($n = 10$)	Age; Gender; Hb genotype; RBCU.	Immune antibodies occurred in 3 Jamaicans (2·6%) and 16 UK subjects (76·0%). Jamaican patients receiving 1–2 units for acute anaemia or ACS, whereas UK patients had multiple BT.	Moderate (6 pts)
20	Rh system (21·0%); Kell system (11·0%); Rh and K system (28·0%)	Rh and K system with others (−M, -N, -S, -Fya, -Fyb, −Jsa, −Jsb, -U) (50·0%)	Age; Gender; History of BT.	Alloimmunisation rate in adults (47·0%) than our paediatric patients (29·0%) with increased prevalence in females.	Moderate (4 pts)
21	14·4% had formed 1 alloantibody; 9% had formed 2;6·3% had formed 3; 2·7% had formed 4; 1·8% had formed ≥5.[3]		Gender.	The alloimmunisation rate was 34·2%.	Moderate (5 pts)

CI, confidence interval; DAT, direct antiglobulin test; DO, demographic origin; ECP, Erythrocytapheresis; G6PD, glucose-6-phosphate dehydrogenase; GO, geographic origin; Hb, haemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, hazard ratio; ICC, immune cell count; LDH, lactate dehidrogenase; *n*, number of observations; *P*, *P* value; pts, points; RBCU, red blood cell units; ST, simple transfusion; TNFA, tumour necrosis factor A; TRV, tricuspid regurgitant jet velocity; WBC, white blood cell.

[1]Methodological quality was evaluated according to NOS (accessed in 2017).

[2]Frequency not reported.

[3]Antibodies not specified.

storage, as a stimulant for the production of alloantibodies by the receiver. However, Nickel *et al.* (2015) and Padmanabhan & Chandrasekaran (2000) mention the receiver's age as a factor directly related to awareness. Higher immune responses to antigens, a greater number of transfusions and the presence of previous pregnancies are some factors that might justify a higher rate of alloimmunisation in older patients (Helman *et al.*, 2011).

Sippert *et al.* (2017) and Tatari-Calderone *et al.* (2016) determined an association of alloimmunisation of the genes in the HLA system. Sippert *et al.* (2017) showed that the presence of some genes' polymorphism could increase the risk of alloimmunisation. Tatari-Calderone *et al.* (2016) found that the HLA-DQ genes in the HLA-DQ2, -DQ3 and -DQ5 alleles and HLA-DQ2/6 combinations and -DQ5/5 were associated with protection against alloimmunisation. HLAs modulate the immune response because they are responsible for presenting antigens (Sippert *et al.*, 2017).

Michot *et al.* (2015) and Wahl *et al.* (2012) performed comparisons between erythrocytapheresis (ECP), where a quantity of blood is taken from the patient and replaced with cells of normal haemoglobin (Kelly *et al.*, 2016), and the technique of conventional BT. They concluded that, despite the individual receiving a quantity of antigens during the automated procedure, this difference in methods did not influence the incidence of alloimmunisation.

Patients 5 years of age or older at their first transfusion were significantly more likely to form antibodies (Sins *et al.*, 2016). The immune response to foreign antigens might be hampered at an early age, or early exposure to RBC antigens might induce immune tolerance to allogeneic antigens (Verduzco & Nathan, 2009). In SCD, progressive splenic dysfunction occurs at a young age, which might impair the spleen's immunoregulatory role and the systemic tolerance to foreign antigens (Bronte & Pittet, 2013).

According to Nickel *et al.* (2015), alloimmunised patients had a significantly increased percentage of CD41 T memory cells compared to non-alloimmunised patients (57% vs 49%, $P = 0{\cdot}0047$), with no other significant differences in the immune cell subsets or laboratory values detected between these groups. Prior murine studies have suggested the relevance of CD41 T cells in RBC alloimmunisation (Hendrickson *et al.*, 2009), suggesting an important role for CD41 T cells in RBC alloimmunity.

The prevention of RBC alloimmunisation remains a major challenge for patients with SCD. All studies reported the use of different transfusion protocols, and Al-Saeed (1997), Bashawri (2007), Natukunda *et al.* (2010), Helman *et al.* (2011), Aly *et al.* (2012), Diarra *et al.* (2013), Fasano *et al.* (2014), Sins *et al.* (2016) and Allali *et al.* (2017) support the importance of the protocol as a protective factor for alloimmunisation.

Although there remains no consistent standard, much effort has been placed on the use of prophylactic antigen matching of units for chronic transfusion recipients. Studies have demonstrated reductions in the incidence of both alloimmunisation and haemolytic transfusion reactions using C, c, E, e, K antigen-matching protocols (Vichinsky *et al.*, 2001; Castro *et al.*, 2002; Lasalle-Williams *et al.*, 2011).

However, caution should be exercised in antigen-matching programmes because there are distinct RBC antigen frequency differences between the donor population, which is predominantly Caucasian, and patients with SCD, who are of predominantly African descent. The most common complete C, c, E, e, K phenotype among African Americans is D+, C−, E−, K−, whereas only approximately 3% of the Caucasian population has this antigen make-up. Additional antigen disparities exist in the MNS, Duffy and Kidd BG systems. The expected result is that alloimmunisation occurs at a higher frequency due to racial antigen disparities (Chou *et al.*, 2013).

Some limitations should be considered in this SRL. The bibliographic search was comprehensive, but many articles were discarded because they do not fit the criteria for eligibility. With this limitation, it is possible that some publications have not been included in this study. Furthermore, as this review is based on published data, it is possible that the publication bias decreases the representativeness of our results.

As strengths, we can cite the selection and evaluation of the methodological quality of the articles by two authors, increasing the likelihood of having identified the publications of interest and having performed a good quality evaluation. Moreover, the development of the study was based on the PRISMA guidelines (Moher *et al.*, 2009), and the methodological quality was determined with NOS (Wells *et al.*, 2013), which is essential for the systematisation and proper understanding of non-randomised studies.

In summary, we verify that even different transfusions protocols did not extinguish alloimmunisation. Therefore, although it is not guaranteed that alloimmunisation will not occur, the use of protocols remains a transfusion security behaviour that is essential to improving the morbidity of individuals with sickle cell anaemia and a chronic need for transfusion. Knowing the variable pattern of the incidence of erythrocyte alloimmunisation, the most common antigens and alloantibodies and the different factors that might be associated with the genesis of this complication, transfusion policies should be based on these characteristics, always remembering the ethnic characteristics and phenotypic compatibility between donors and patients with SCD. We suggest a protocol for the implementation of phenothiazine bags, a blood bank of rare donors, at the sectoral level.

What is known about the topic?

- Transfusion therapy is a common practice in the treatment of anaemia that can cause the erythrocyte alloimmunisation.

What is new?

- The risk factors for alloimmunisation were age; gender (female); RBC units received; presence of ≥1 autoantibodies, TNF-α, IL1B, HLA-DRB1 gene polymorphisms; first BT after 5 years old, transfusion episodic, multiple or during inflammatory events, ACS and VOC; increased percentage of CD41 T memory cells; and positive direct antiglobulin test.

What are the key questions for future work on the topic?

- Transfusion policies should be developed to protect the patient and the modification of the clinical outcomes of their health based on the main factors associated with its incidence.

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CONFLICT OF INTEREST

The authors have no competing interests.

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