Red cell alloimmunization in sickle-cell anaemia patients

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التمنيع الـمُخَايِف للكريات الحمر في مرضى فقر الدم المنجلي

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الخلاصة: قيَّمت الباحثة في هذه الدراسة التي أُجريَت في مستشفى الملك فهد الجامعي في المملكة العربية السعودية، معدل تواتر التمنيع الـمُخَايِف لمستضدات الكريات الحمر لدى مرضى فقر الدم المنجلي خلال الفترة 1996–2004، وذلك لتقييم اختطار التمنيع الـمُخَايِف والتعرف على أكثر الأضداد الـمُخَايِفة شيوعاً. وقد أظهرت التحاليل الاستعادية لسوابق نقل الدم والسجلات الطبية لـ 350 مريضاً تتراوح أعمارهم بين 2 و75 عاماً، ممن تلقّوا نقلاً للدم لمرة واحدة على الأقل، على أن 48 مريضاً كانت لديهم أضداد مُخَايِفة (13.7٪)، وأن أكثر الأضداد الـمُخَايِفة المكتشفة شيوعاً هي أضداد – ئي وحدها في 18.8٪، وأضداد لا نوعية في 12.5٪، وأضداد غير حصرية في 12.5٪، وأضداد – ك في 10.4٪ وأضداد C-3 في 6.3٪. في حين كان لدى بعض المرضى واحد من الأضداد فقط، كان لدى بعضهم الآخر أكثر من ضد واحد، وظلت نتائج اختبار أضداد الغلوبولين المباشرة إيجابية لدى تسعة من المرضى.

ABSTRACT This study in King Fahd Hospital of the University, Saudi Arabia, assessed the frequency of alloimmunization to red cell antigens in sickle-cell anaemia patients over 1996–2004 in order to evaluate the risk of alloimmunization and identify the most common alloantibodies. A retrospective analysis of the transfusion history and medical records of 350 patients aged 2 to 75 years who had received at least 1 transfusion found that 48 patients had developed alloantibodies (13.7%). The most common alloantibodies detected were: anti-E alone (18.8%), nonspecific (12.5%), inconclusive (12.5%), anti-K (10.4%) and anti-c 3 (6.3%). Some patients had 1 alloantibody, while others more than 1 and even multiple antibodies. Nine patients had a persistent positive direct antiglobulin test.

Allo-immunisation anti-érythrocytaire dans la drépanocytose

RÉSUMÉ Cette étude, menée de 1996 à 2004 en Arabie saoudite à l'Hôpital universitaire du Roi Fahd, a évalué la fréquence de l'allo-immunisation contre les antigènes érythrocytaires chez des patients atteints de drépanocytose (ou anémie falciforme) dans le but d'évaluer le risque d'allo-immunisation et d'identifier les alloanticorps les plus communs. Une analyse rétrospective de l'histoire transfusionnelle et du dossier médical de 350 patients âgés de 2 à 75 ans ayant reçu au moins une transfusion a révélé que 48 de ces patients avaient développé des alloanticorps (13,7 %). Les alloanticorps les plus fréquemment détectés appartenaient aux catégories suivantes : anti-E (18,8 %), non spécifiques (12,5 %), indéterminés (12,5 %), anti-K (10,4 %) et anti-c3 (6,3 %). Certains patients ne présentaient qu'un seul alloanticorps, tandis que chez d'autres on en a identifié plusieurs, voire une multiplicité d'alloanticorps. Dans neuf cas, on a pu constater la persistance de la positivité du test direct à l'antiglobuline (TDA), ou test direct de Coombs.

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Introduction

Red blood cell (RBC) transfusions are frequently used in sickle-cell anaemia (SCA) patients to treat and prevent the complications of their disease. Acute simple transfusions are usually used to treat sequestration crisis, aplastic crisis, blood loss and in preoperative preparation. Chronic transfusion therapy, which is being used with increasing frequency, is recommended for patients who have cerebrovascular disease, complicated pregnancy, cardiopulmonary disease and severe debilitating vaso-occlusive disorders. Exchange transfusion is needed in cerebrovascular disease, priapism, hepatic failure and also in preoperative preparation [*1–3*].

Unfortunately, while transfusions are needed and effective in preventing morbidity and mortality from SCA, their use is complicated by a high incidence of RBC alloimmunization and other transfusion related complications [*1–7*]. The incidence of RBC alloimmunization in SCA patients has been reported to range between 8% to 50% [*2,8*], with an average range of 20%–30% [*2,7,8*]. All physicians, haematologists and transfusion specialists who participate in the management and transfusion of SCA patients know the problems associated with alloimmunization, mainly the difficulty in finding compatible blood for these patients and the delayed haemolytic transfusion reactions (DHTRs) that may occur.

The most common alloantibodies reported to be detected include in rank order: anti-E, anti-C, anti-K, followed by anti-Fy^a, anti-JK^b, anti-S and anti-D [*1–9*]. Many patients develop multiple alloantibodies, which further complicate their situation. Up to a third of antibodies are transitory and may not be detected during pretransfusion testing, so DHTR can occur. The reported incidence of DHTR is around 11% [*4,10*]. It should be remembered also that DHTR may not be diagnosed, since the symptoms can mimic the features and complications of SCA, i.e. vaso-occlusive crisis and haemolytic crisis [*4,10*].

Many studies have shown the importance of providing antigen-matched blood for chronic transfusion patients, such as those with thalassaemia and SCA, in order to decrease the frequency of alloimmunization and its related complications [*2,11,12*]. There have also been a number of research reports exploring the effect of transfusion from different ethnic and racial groups and the variability in rates of alloimmunization that can occur. Racial mismatch is believed to be one of the reasons for the high level of alloimmunization in SCA patients [*6,13–15*]. The main objective of this study was to assess the frequency of alloimmunization over a 9-year period in SCA patients managed in a university hospital in Saudi Arabia, in order to provide appropriate recommendations for the care of these patients.

Methods

A retrospective analysis was made from 1 January 1996 to 31 December 2004 of the transfusion history and medical files of 350 patients with SCA. Patients were those aged 2 to 75 years who had received at least 1 transfusion with units of ABO and D matched RBCs at King Fahd Hospital of the University, Dammam, Saudi Arabia.

Laboratory investigations

After ABO and Rh blood grouping by the standard tube method, the following were routinely done for every patient:

- Antibody screening. Prior to every transfusion, sera were tested for the presence of alloantibodies using a 2-cell panel with homozygous expression of the antigens (Ortho-Clinical Diagnostics, USA.

and DiaMed, ID-Micro typing system, Morat, Switzerland). Any pretransfusion sera with a positive antibody screen were subjected to antibody identification.

- Antibody identification. Antibody specificity was performed using a commercial RBC panel with known antigens (Resolve Panel A and B, Ortho Clinical Diagnostics, USA), and DiaMed ID-DIA panel (without enzymes) and ID-Dia Panel P (with enzymes) against the patient's serum.
- Direct antiglobulin test (DAT). This was performed using 3%–5% of patient's RBCs and appropriate controls. A polyspecific antiglobulin reagent was used. The results were read macroscopically and microscopically, and all negative results were confirmed by adding control cells.
- Crossmatch. A crossmatch was performed prior to transfusion, after the selection of the appropriate blood from the blood group results, antibody screening and identification. First a saline procedure was performed at room temperature, then at 37 ºC, followed by an antiglobulin phase using albumin. Blood lacking the antigen for the corresponding alloantibody identified was chosen and blood was transfused if crossmatch was compatible (i.e. antigen-negative, crossmatch-compatible blood).

In addition, 126 healthy blood donors of Saudi Arabian nationality were phenotyped for Rh and K antigens by the card method (DiaMed) according to the manufacturer's instructions.

Results

A total of 48 patients developed alloantibodies (13.7%). The mean age was 28.8 (SD 15.0) years, and there were 25 females and 23 males. The most common blood groups were in rank order: O+ (n = 25 patients), A+ (n = 7), B+ (n = 12), AB+ (n = 3) and B– (n = 1). The mean haemoglobin S level was 80.0% (SD 12.0%), mean haemoglobin F was 17.3% (SD 10.1%) and mean haemoglobin A2 was 1.50% (SD 0.85%).

Table 1 shows the specificities of the identified alloantibodies as well as patients' data. Table 2 shows the alloantibodies detected in the different patients. The most common alloantibodies detected were, in rank order: anti-E alone (n = 9 patients, 18.8%), nonspecific (n = 6, 12.5%), inconclusive (n = 6, 12.5%), anti-K (n = 5, 10.4%) and anti-c (n = 3, 6.3%). Some patients had 1 alloantibody, while others more than 1 and even multiple antibodies. Table 3 shows the frequency of the different alloantibodies found in the study.

Nine (9) patients had a persistent positive DAT (Table 1), but with no evidence of autoimmune haemolytic anaemia.

The results of the donor phenotyping for Rh and Kell antigens showed that the most common Rh phenotypes among the 126 donors were in rank order: CcDe (n = 49, 38.9%), CDe (n = 21, 16.7%), dce (n = 17, 13.5%), CDEe (n = 16, 12.7%), cDe (n = 11, 8.7%), CcDEe (n = 9, 7.1%), cDE (n = 2, 1.6%) and CcDE (n = 1, 0.8%). For the Kell antigen 110 blood donors were K negative and 16 were K positive, suggesting the possible phenotypes to be K– k+ and K+ k+ respectively, since the K+ k– phenotype is rare.

Discussion

Transfusion therapy is the mainstay of treatment for patients with SCA. Blood banks play a key role in the delivery of this care. Actual blood bank policies and practices for SCA patients, however, are largely un-

Table 1 **Profile of sickle-cell anaemia patients who developed alloantibodies, according to King Fahd Hospital of the University blood bank and hospital records (transfusions elsewhere cannot be excluded)**

Patient no.	Sex	Age (years)	Blood group	DAT	Alloantibodies	No. of blood transfusions (donor nationality)
1	n/a	37	O+	–ve	Anti-K and nonspecific	6 (Saudi)
2	F	19	O+	–ve	Anti-Fy^a, anti-E	16 (14, Saudi, 1 Yemeni, 1 Filipino)
3	F	32	B+	–ve	Anti-K	4 (Saudi)
4	F	16	A+	–ve	Anti-c, anti-K	4 (Saudi)
5	M	21	A+	–ve	Anti-E	2 (Saudi)
6	M	59	O+	+ve	Anti-K, anti-S, nonspecific	11 (8 Saudi, 1 Pakistani, 2 Indian)
7	M	16	B+	–ve	Anti-E	2 (Saudi)
8	F	26	B+	–ve	Anti-E	4 (Saudi)
9	M	33	AB+	–ve	Anti-K	4 (2 Saudi, 1 Syrian, 1 Egyptian)
10	M	19	O+	–ve	Nonspecific and anti-E	12 (11 Saudi, 1 Pakistani)
11	F	32	O+	–ve	Anti-K	6 (Saudi)
12	F	15	O+	–ve	Anti-M and multiple nonspecific	8 (7 Saudi, 1 Syrian)
13	F	11	A+	–ve	Nonspecific	2 (Saudi)
14	F	27	B+	–ve	Anti-c and other nonspecific	4 (2 Saudi, 1 Indian, 1 Bangladeshi)
15	M	11	A+	–ve	Nonspecific	11 (8 Saudi, 1 Pakistani, 1 Sudanese, 1 Jordanian)
16	M	9	O+	–ve	Nonspecific	7 (6 Saudi, 1 Palestinian)
17	M	10	A+	–ve	Inconclusive	2 (1 Saudi, 1 Egyptian)
18	F	16	B-	+ve	Anti-D, Anti-c	3 (Saudi)
19	F	25	O+	–ve	Anti-K	2 (Saudi)
20	M	48	AB+	+ve	Inconclusive	1 (Saudi)
21	F	20	B+	–ve	Anti-c, anti-E and nonspecific	2 (Saudi)
22	M	63	O+	–ve	Anti-c	1 (Saudi)
23	F	25	O+	+ve	Anti-JK^a, anti-C, anti-E, anti-K, anti-Lu^a and nonspecific	24 (21 Saudi, 1 Egyptian, 1 Yemeni, 1 Sudanese)
24	M	7	B+	–ve	Anti-E	16 (15 Saudi, 1 Indian)
25	F	41	O+	–ve	Anti-K, anti-JK^a	2 (Saudi)
26	F	55	B+	+ve	Anti-c and nonspecific	1 (Saudi)

Table 1 **Profile of sickle-cell anaemia patients who developed alloantibodies, according to King Fahd Hospital of the University blood bank and hospital records (transfusions elsewhere cannot be excluded)** *(concluded)*

Patient no.	Sex	Age (years)	Blood group	DAT	Alloantibodies	No. of blood transfusions (donor nationality)
27	M	21	O+	–ve	Anti-E and nonspecific	11 (Saudi)
28	F	41	B+	–ve	Anti-E	12 (Saudi)
29	F	29	O+	–ve	Anti-K	17 (13 Saudi, 1 Filipino, 2 Sudanese, 1 Indonesian)
30	M	25	O+	–ve	Anti-c	4 (3 Saudi, 1 Indian)
31	F	31	O+	–ve	Anti-E, anti-K, anti-Fy^a, anti-Fy^b, anti-Lu^a , anti-S and inconclusive	20 (16 Saudi, 1 Bahraini, 2 Indian, 1 Syrian)
32	M	17	A+	–ve	Anti-E, anti-C, anti-JK^b, anti-C^w and inconclusive	10 (7 Saudi, 1 Indian, 1 Jordanian, 1 Sudanese)
33	F	41	O+	–ve	Anti-K and nonspecific	11 (8 Saudi, 3 non Saudi)
34	F	22	B+	–ve	Anti-E	2 (Saudi)
35	F	29	O+	–ve	Anti-E	5 (2 Saudi, 2 Yemeni, 1 Indian)
36	M	75	O+	–ve	Anti-E	3 (Saudi)
37	F	17	B+	–ve	Inconclusive	6 (5 Saudi, 1 Pakistani)
38	F	24	O+	+ve	Inconclusive	2 (1 Saudi, 1 Jordanian)
39	F	30	B+	–ve	Anti-C, anti-E, anti-C^w and nonspecific	4 (Saudi)
40	F	27	B+	–ve	Anti-E	2 (Saudi)
41	M	63	O+	–ve	Anti-c	3 (Saudi)
42	M	19	A+	+ve	Nonspecific	14 (9 Saudi, 1 Bahraini, 1 Syrian, 1 Sudanese, 1 Egyptian, 1 Yemeni)
43	M	32	O+	–ve	Nonspecific	5 (3 Saudi, 1 Egyptian, 1 Yemeni)
44	M	36	AB+	+ve	Anti-c, anti-E	4 (Saudi)
45	F	29	O+	+ve	Nonspecific and anti-c	3 (2 Saudi, 1 Indian)
46	M	23	O+	–ve	Inconclusive	2 (Saudi)
47	M	31	O+	–ve	Inconclusive	4 (Saudi)
48	M	26	O+	–ve	Nonspecific	1 (Saudi)

DAT = direct antiglobulin test.
n/a = information unavailable, –ve = negative, +ve = positive.

Table 2 **Blood group antibodies detected in 48 sickle-cell anaemia patients who developed alloantibodies**

Antibody	No. of patients
E	9
Nonspecific	6
Inconclusive[b]	6
K	5
c	3
c and nonspecific	3
c, E	2
E and nonspecific	2
K and nonspecific	2
Fy[a], E	1
K, S and nonspecific	1
M and nonspecific	1
D, c	1
c, E and nonspecific	1
JK[a], C, E, K, Lu[b] and nonspecific	1
E, JK[a]	1
E, K, Fy[a], Fy[b], Lu[a], S and nonspecific	1
E, C, JK[b], C[w] and nonspecific	1
C, E, C[w] and nonspecific	1

14 patients had more than 1 alloantibody.
Inconclusive indicates unidentified, specificity indeterminate.

Table 3 **Alloantibody prevalence in the 48 sickle-cell anaemia patients who developed alloantibodies**

Alloantibody	Prevalence %
Anti-E	42
Nonspecific	38
Anti-c	21
Anti-K	21
Inconclusive	17
Anti-C	6
Anti-S	4
Anti-Fy[a]	4
Anti-JK[a]	4
Anti-C[w]	4
Anti-Fy[b]	2
Anti-M	2
Anti-D	2
Anti-JK[b]	2
Anti-Lu[a]	2
Anti-Lu[b]	2

Inconclusive indicates unidentified, specificity indeterminate.

known in Saudi Arabia. Haematologists and physicians managing SCA patients understand the importance of simple, exchange and chronic transfusion in the management of these patients. As many SCA patients develop alloantibodies, the procurement of compatible units may be very difficult in the long run. In this study 48 out of 350 SCA patients (13.7%) formed clinically significant alloantibodies. This is within the range of the alloimmunization rate reported in the literature [*2,8*]. Another study from the eastern province of Saudi Arabia on SCA patients revealed an alloimmunization rate of 34.2%, where 38 out of 111 patients developed alloantibodies [*7*].

In this present study alloantibodies to E and c of the Rh system and to the K antigen were most commonly encountered. These alloantibodies have been the most commonly detected in many reports [*1–9*]. This is why it has often been advocated that even partial red blood cell matching for at least Rh and Kell antigens should be done to reduce the rate of alloimmunization; however, the risk of alloimmunization to unmatched antigens still exists. Reports of performing just partial phenotype matching to Rh and Kell antigens have proved to be useful and effective in decreasing the incidence of alloimmunization [*11,12,16,17*]. Even in our

study many patients developed more than one alloantibody, which increases the difficulty in finding compatible blood. In our study and the study by Al Saeed [*7*] anti-E was detected most frequently, appearing alone or in combination with other alloantibodies than anti-c. However, in other studies anti-C appeared more often than anti-c [*11,16*], again showing the differences in distribution of RBC antigens. Alloantibodies to other blood group systems commonly detected were also found in our study, i.e. Kidd (Jk^a, Jk^b), Duffy (Fy^a, Fy^b), Lutheran (Lu^a, Lu^b) and MNSs.

The clinical and laboratory consequences of alloimmunization—i.e. delayed and/or immediate haemolytic reactions as well as the "laboratory stress" in obtaining compatible blood especially for those patients who have already developed an alloantibody or multiple antibodies—warrants adopting a policy for routine RBC antigen matching in all SCA patients. This will prevent alloimmunization, as shown by many studies [*11,16,17*]. This issue has been controversial and vigorously debated. The main drawbacks are the costs, the time and labour involved in performing extended and even partial RBC phenotyping. However, experience has demonstrated that providing antigen-matched blood prevents alloimmunization as well as other complications for these patients that may outweigh the higher costs of the process [*11,12*]. Patients with multiple alloantibodies pose great difficulties for hospitals in finding compatible blood. This is very critical for these patients as some of them present in emergency situations as well as presenting to other hospitals for the first time, complicating the matter even further.

The issue of blood transfusion between races and race-related alloimmunization, which is well documented in different studies [*6,13–15*], has not been studied in Arab donors and recipients. For example, most donors in the United States of America are Caucasian and SCA recipients are almost exclusively of African ancestry; this has been reported to be a cause for a high rate alloimmunization in these patients [*13*]. Furthermore the greater red cell alloimmunization reported among United Kingdom (UK) SCA patients reflects the racial disparity between donor and recipient populations in the UK as well as greater use of transfusions [*15*]. In our study most of the blood was provided from Saudi blood donors. However, some blood units were provided from blood donors of other nationalities (especially during times of blood shortage or rare blood groups), such as Pakistani, Indian, Indonesian, Filipino, Bangladeshi and other Arabs (Yemeni, Bahraini, Egyptian, Syrian, Palestinian, Jordanian and Sudanese). It was noticed that some patients who had increased transfusion requirements and who have received blood from different nationalities tend to develop multiple alloantibodies as well as nonspecific antibodies. But this has to be studied further and substantiated with further transfusion analysis of these patients. To get an idea of the distribution of Rh and K antigens, we phenotyped 126 Saudi blood donors and found the most common Rh phenotype to be CcDe, which follows what has been previously reported [*18*]. Concerning the Kell phenotype, the majority were found to be K negative, suggesting that the most common Kell phenotype to be the heterozygous Kell phenotype Kk (K– k+) as reported in the literature [*19*]. So finding antigen-matched blood at least to Rh and Kell antigens should not be that difficult.

The DAT was positive in 9 patients, without evidence of autoimmune haemolysis; the significance of this is still unclear. The phenomenon of development of autoimmunization as a result of frequent blood

transfusions and the presence of alloantibodies has been known for many years, but has received little attention [*20,21*]. Leukocyte-depleted blood is also recommended for these patients as studies have shown that storage of RBCs at 1–6 ºC will induce apoptosis in white blood cells (WBCs) leading to release of immunostimulatory antigens and soluble biologic mediators from dying cells which may sensitize the immune system of transfusion recipients and therefore lead to autoimmune disease [*22*]. This may explain the phenomenon of autoimmunization mentioned above. It should also be remembered that alloimmunization to transfused blood cells is not limited to RBC antigens; alloimmunization to WBC and platelet antigens can occur in SCA patients [*23*], which may cause further problems in the future. For example, recurrent febrile nonhaemolytic transfusion reactions and platelet refractoriness, a condition that would increase their risk if bone marrow transplantation, were considered. Again, this emphasizes the importance of using leukocyte-reduced blood for these patients. We should also not forget the other complications of alloimmunization. For example, after alloimmunization haemolytic transfusion reactions and even hyperhaemolysis may occur and be mistaken for sickle cell crises since differentiation of these 2 entities constitutes a problem in the management of these patients [*5,10,17*].

SCA patients deserve special transfusion practices. Further properly designed conducted clinical trials are needed to provide sound information for optimal transfusion policies. Certain recommendations concerning transfusion support need to be implemented for the benefit of these patients:

1. Transfusion of patients at least with red cells matched for the main Rh antigens and Kell antigen (partial antigen matching) if extended matching cannot be done.
2. Transfusion of leukocyte-reduced blood as well as sickle-cell negative blood.
3. Provision of clear national transfusion procedures and policies for all SCA patients to provide overall optimal care including preoperative guidelines.
4. Instigating studies to determine the distribution of the clinically relevant blood group antigens in the Saudi population.

In conclusion, the issue of alloimmunization and its consequences is important for both the clinical management and laboratory practice of SCA patients.

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