Conservation of the group O, Rhesus D negative blood supply

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The overall health of blood supply in countries with developed care systems is increasingly challenged by shifting use practices. For example, a decline in demand has occurred for red cells in general populations, but has been accompanied by a concomitant increase in demand for group O, Rhesus D negative [RhD–] blood for management of massive haemorrhage and other urgent transfusion scenarios. Moreover, newly identified infectious diseases pose a risk for transfusion-transmitted disease (eg, Zika virus), and the donor population is ageing.[1–3] Yet there is a common expectation that blood will always be available in times of need. This expectation is particularly challenging for the so-called universal blood products—ie, those that can be transfused to any patient, irrespective of ABO and Rhesus D blood type: group O, RhD– red cells and group AB plasma. Focusing on red cells only, the group O, RhD– donor represents only 8% of the overall blood donor population. However, based on recent blood centre data from the USA and Australia, roughly 10·5% of red blood cells distributed are group O, RhD–.[1] Thus, a scenario is possible in which the supply of this particular blood component could rapidly dwindle in the face of ongoing indiscriminate and liberal transfusion practice.

Although use of group O red cells for urgent transfusion is prudent to avoid risk of ABO incompatible haemolytic transfusion reactions, the use of group O, RhD– red cells to avoid either haemolytic transfusion reactions in patients previously alloimmunised to the D antigen (but not known at the time of urgent transfusion request) or to prevent sensitisation to the D antigen is not necessary. Several reports[4,5] have shown that the risk for haemolytic reactions due to unknown antibodies at the time of urgent transfusion is extremely low (~0·4% of emergency transfusion episodes), supporting the safety of transfusion of group O, Rhesus D positive (RhD+) units followed by ABO, RhD type specific units. Moreover, because 85% of the recipient population is RhD+, issuing group O, RhD– red cells to patients with unknown blood type to mitigate risk of alloimmunisation is unnecessary.[6]

A report by Kathleen Selleng and colleagues[7] in *The Lancet Haematology* highlights the potential positive impact of a novel transfusion strategy in patients requiring urgent or emergency transfusion in the setting of unknown blood type on conservation of group O, RhD– red cells. Acknowledging that roughly 85% of the recipient patient population is RhD+, and thus not a risk for alloimmunisation or the possible associated downstream complications, the investigators employed a transfusion protocol in which patients with unknown blood type in need of urgent or emergency transfusion received group O, RhD+ red cells, followed by ABO, identical RhD+ red cells. Not only did they observe a relatively low risk for RhD– alloimmunisation with employment of this protocol compared with RhD– patients who received RhD+ red cells due to inventory shortages (17 [4%] of 437 patients *vs* 29 [26%] of 110 patients; p<0·0001), but also application of the protocol resulted in the preservation of almost 10% of their group O, RhD– red cell inventory for use in RhD– patients.

In consideration of the fragility of the blood supply, in particular the supply of group O, RhD– red cells, Selleng and colleagues[7] are to be commended for their alternative transfusion strategy for urgent or emergency transfusion in the setting of unknown blood type. Other groups have similarly shown that more than 15% of group O, RhD– red cells were issued to patients in the setting of unknown blood type or trauma who were later determined to be RhD+.[3] Conservation of these units would greatly benefit known RhD– patients requiring transfusion. Perhaps this finding is the necessary justification for national and international adoption of a transfusion policy in which group O, RhD+ red cells are used in favour of group O, RhD– red cells for urgent or emergency transfusion when the blood type is unknown.

Other measures directed at conserving this precious resource should also be considered. For example, use of RhD type-specific blood for neonatal transfusion; discouragement of the use of group O, RhD– red cells for ease of transfusion in patients with alloantibodies to other Rh system antibodies (eg, use of RhD– units in an RhD+ patient with allo-E antibodies); and more extensive antigen typing of group A and other blood group red cells which could be used in place of group O red cells. After all, provision of phenotypically compatible blood—group O, RhD– red cells to the group O, RhD– recipient—is the essence of personalised medicine.[8]

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I declare no competing interests.

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Emergency transfusion of patients with unknown blood type with blood group O Rhesus D positive red blood cell concentrates: a prospective, single-centre, observational study

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Summary

Background Emergency patients with unknown blood type usually receive O Rhesus D negative (RhD–) red blood cell concentrates until their blood group is determined to prevent RhD+ related adverse transfusion reactions. As 85% of individuals are RhD+, this consumption of O RhD– red blood cell concentrates contributes to shortages of O RhD– red blood cell concentrates, sometimes forcing transfusion of known RhD– patients with RhD+ red blood cell concentrates. Here we report the outcome of this transfusion policy transfusing all emergency patients with unknown blood type with O RhD+ red blood cell concentrates.

Methods In this prospective single-centre observational study done between Jan 1, 2001, and Dec 31, 2015, we assessed all consecutive RhD– patients at the University Medicine Greifswald who received RhD+ red blood cell concentrates (emergency patients with unknown blood type; and RhD– patients receiving RhD+ red blood cell concentrates during RhD– red blood cell concentrate shortages). No patients were excluded. The primary endpoint was anti-D allo-immunisation at 2 months follow-up or later. Patients were followed up and tested for immunisation against red blood cell antigens using the direct antiglobulin test and an antibody screen every 3–5 days for 4 weeks or until death, or hospital discharge. Surviving patients were screened for development of anti-D antibodies for up to 12 months (at the predefined timepoints 2, 3, 6, and 12 months) after RhD+ red blood cell transfusion.

Findings 437 emergency patients, of whom 85 (20%) were RhD–, received 2836 RhD+ red blood cell concentrates. The overall risk of inducing anti-D antibodies (in all 437 recipients) was 17 (4%, 95% CI 2·44–6·14) of 437 (assuming all patients lost to follow-up developed anti-D allo-immunisation). During this period, 110 known RhD– patients received RhD+ red blood cell concentrates during RhD– red blood cell concentrate shortages. Of these, 29 (26%; 95% CI 19·0–35·3) developed anti-D allo-immunisation (assuming all patients lost to follow-up developed anti-D), which was significantly higher than in the emergency patients with unknown blood type (p<0·0001).

Interpretation Transfusing emergency patients with unknown blood type with O RhD+ red blood cell concentrates has a low risk of inducing anti-D antibodies (3–6%), but saves more than 10% of the total O RhD– red blood cell concentrate demand, thereby reducing shortage of O RhD– red blood cell concentrates, the need to transfuse known RhD–patients with RhD+ red blood cell concentrates, and thus the overall risk to induce anti-D allo-immunisation in the population. These findings should be considered for transfusion guidelines.

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Introduction

The most important blood groups for red blood cell concentrate transfusion are ABO and Rhesus D. Individuals with the blood group O Rhesus D negative (RhD–) are considered universal donors as their red blood cells do not induce acute haemolytic transfusion reactions due to ABO incompatibility and do not raise the risk for adverse transfusion reactions due to anti-D allo-immunisation.

Providing ABO– and Rhesus-specific red blood cell concentrates is sometimes not feasible when patients are admitted to the emergency room with massive bleeding. In this situation, transfusion of RhD+ red blood cell concentrates in potentially RhD– patients bears the risks of formation of an anti-D allo-antibody within 3–12 weeks after transfusion, which occurs in about 10–30% of cases;[1–3] boosting of a previously acquired anti-D immune response (eg, during pregnancy) with formation of high titre anti-D IgG antibodies within 5–10 days, which bind to the transfused RhD+ red blood cells causing delayed haemolytic transfusion reaction;[4] induction of an acute haemolytic transfusion reaction, if high levels of preformed anti-D are present in the patient's plasma; and in girls and women of childbearing age, immunisation against RhD+ can result in severe haemolytic disease in the fetus during subsequent pregnancies.

Although some major trauma centres often use O RhD+ red blood cell concentrates in men in emergency situations, it is still common practice in many hospitals to transfuse patients who require emergency transfusion

Research in context

We searched PubMed for all English language articles published between Jan 1, 1970, and Jan 31, 2017, using the search terms "anti-D", "Rhesus incompatible transfusion", "Rhesus positive red cell concentrate transfusion in Rhesus negative patients", "Rh+ red cell concentrate transfusion in Rh– patients", "Rh incompatible erythrocyte/RBC transfusion", and "emergency transfusion". We found no prospective randomised trials. A retrospective analysis of a transfusion strategy in which patients were transfused with blood group O Rhesus D negative (RhD–) red blood cell concentrates, followed by Rhesus D positive (RhD+) red blood cell concentrates in RhD+ patients or in males, described 268 patients of whom 18 RhD– patients received RhD+ red blood cell concentrates. In this analysis, only one patient developed anti-D antibodies (0·4% of all patients and 5·6% of RhD– patients). A second retrospective analysis found an anti-D immune response in 16 of 78 RhD– patients receiving RhD+ red blood cell concentrates, with a calculated probability of developing anti-D below 42% (upper 95% confidence bound) and estimated as 30%. A third retrospective analysis of 98 RhD– patients who received a total of 445 RhD+ red blood cell units identified 22 (22%) of 98 patients who developed anti-D antibodies. A prospective study, which analysed the use of RhD+ red blood cell concentrates in 351 RhD– patients also found an incidence of anti-D allo-immunisation in 21·4% of patients, whereas a second prospective study found development of anti-D antibodies in three (12%) of 26 RhD– patients receiving RhD+ red blood cell concentrates during times of RhD– red blood cell concentrate shortages. We found no other studies assessing a transfusion policy of transfusing all patients with unknown blood type who required emergency (massive) transfusions with O RhD+ red blood cell concentrates.

Although some major trauma centres used O RhD+ red blood cell concentrates in men with unknown blood type in emergency situations, many hospitals use the transfusion policy to transfuse patients who required emergency transfusion with 4–6 O RhD– red blood cell concentrates until the blood group had been determined. A survey involving six European level one trauma centres showed that initial transfusion of O RhD– red blood cell concentrates in patients with unknown blood type is clinical practice in all six centres.

Added value of this study

To determine the overall risk for induction of anti-D allo-immunisation at the time the decision for the emergency transfusion has to be made, the entire population of patients receiving the emergency transfusion should be considered, and not only RhD– patients who ultimately survive. To our knowledge, this study is the first to address this overall risk.

Implications of all the available evidence

The data provided by the study suggest that transfusion guidelines should recommend the use of blood group O red blood cell concentrates in emergency transfusion without considering the Rhesus blood group.

with 4–6 O RhD– red blood cell concentrates until the blood group has been determined.[5–7] Indeed, a survey involving six European level one trauma centres found that initial transfusion of O RhD– red blood cell concentrates in patients with unknown blood type is standard clinical practice in all six centres.[7] After blood group typing, further red blood cell transfusions are selected according to the patient's ABO and RhD type.

Only 6–8% of the blood donor population have the blood group O RhD–,[8] whereas most (emergency) patients are RhD+ (roughly 85%).[8] The use of O RhD– red blood cell concentrates as universal blood therefore leads to an over-proportionately high consumption of O RhD– red blood cell concentrates and, consequently, increases the risk that shortages of O RhD– red blood cell concentrates occur. This is exemplified by two studies, one in the USA and one in Australia, in which 6·3–7·4% of first time donors had blood group O RhD–, but 10·5–13·9% of all distributed red blood cell concentrates were blood group O RhD–.[9] The limited availability of O RhD– red blood cell concentrates already forces blood banks to issue RhD+ red blood cell concentrates for transfusion of RhD– male patients and female patients beyond childbearing capacity,[1,10] and 20–30% of them will develop anti-D antibodies.[1,2,11]

We and others[5,12] therefore consider that the use of O RhD+ red blood cell concentrates for emergency transfusions of patients with unknown blood type reduces the risk that shortages of O RhD– red blood cell concentrates develop. This will reduce situations requiring transfusion of known RhD– patients with RhD+ red blood cell concentrates, and therefore the risk for anti-D allo-immunisation in the overall RhD– population. Only sparse data are available about the consequences of such a policy, partly because transfusion of RhD– patients with RhD+ red blood cell concentrates is an infrequent event, making outcome studies difficult. Here we report the outcome of this transfusion policy at a single centre, including all consecutive patients who received O RhD+ red blood cell concentrates for emergency transfusions, and all patients known to be RhD– who received RhD+ red blood cell concentrates due to shortage of RhD– red blood cell concentrates over a 15-year period.

Methods

Study design and patients

In this prospective, observational study, done between Jan 1, 2001, to Dec 31, 2015, at the University Medicine Greifswald, all consecutive patients with unknown blood

type were included (without any exclusion) who received emergency transfusions of O RhD+ red blood cell concentrates. When the blood type had been determined, patients were further transfused with ABO identical and RhD+ red blood cell concentrates.

For urgent emergencies (transfusion requirement within 10–20 min, a timeframe which usually does not permit pre-transfusion blood group typing), we maintain a depot of pre-documented, ready-to-transfuse red blood cell concentrates of blood group O RhD+, which can be accessed by personnel of the emergency department.

Beside emergency patients with unknown blood type, all RhD– patients with known RhD– blood type who had been transfused with O RhD+ red blood cell concentrates because of RhD– red blood cell concentrate shortages during the same observation period were enrolled. These patients received RhD+ red blood cell concentrates when their transfusion demand exceeded the minimum stock of RhD– red blood cell concentrates in the blood bank. All patients received in-line leucocyte-depleted red blood cell concentrates. The red blood cell concentrate production method did not change during the study period. Beside red blood cells, patients might have received other blood products including factor concentrates as required by the clinical situation.

The study design and protocol as a quality measure of transfusion practice had been approved by the institutional ethics review board of the University Medicine Greifswald. Because of the nature of the study on emergency transfusions, the ethics review board agreed that obtaining consent before enrolment would not have been possible.

Procedures

Patients were followed up and tested for immunisation against red blood cell antigens using the direct antiglobulin test and an antibody screen every 3–5 days for 4 weeks or until death, or hospital discharge. Surviving patients were screened for development of anti-D for up to 12 months (at the predefined timepoints 2, 3, 6, and 12 months) after RhD+ red blood cell transfusion. Two patients who missed the follow-up schedule were retested for anti-D more than 1 year after the RhD+ red blood cell transfusion. Charts of patients who developed anti-D within 4 weeks after the RhD+ red blood cell transfusion were screened for laboratory signs of haemolysis (bilirubin, lactate dehydrogenase, haptoglobin, or haemoglobin), or clinical symptoms or signs of a delayed haemolytic transfusion reaction, assessed independently by at least two of the authors (SS and KS or BM and KS). In case of disagreement, a third independent opinion (AG) was obtained and final consensus achieved by the three adjudicators. Antibody screening, direct antiglobulin test and differentiation, and low pH antibody elution from the patient's autologous red blood cells were done using standard blood bank techniques.

We did a post-hoc analysis to compare the mortality of RhD+ and RhD– patients who had been assessed in a retrospective study of blood use and 5-day and 30-day survival at 11 hospitals in six nations between 2009 and 2013.[10] Of 1360 enrolled patients who received a massive transfusion (transfused with 20 or more red blood cell units over the course of any 2 consecutive calendar days), information about the RhD blood type of the patient and the transfused red blood cell concentrates was available for 863 patients. These 863 patients were used for the post-hoc analysis.

Outcomes

The primary endpoint was development of anti-D antibodies at 2 months follow-up or later. Secondary endpoints were mortality, and signs and symptoms of acute or delayed transfusion reactions up to 4 weeks after RhD+ red blood cell emergency transfusion.

Statistical analysis

Comparison between groups was done by Fisher's exact test. The odds ratio (OR) for the risk of mortality and the 95% CI for the risk of inducing anti-D allo-immunisation were calculated using SAS version 9.3.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. KS, GJ, AG had full access to all the data in the study. The corresponding author had final responsibility for the decision to submit for publication.

Results

437 patients received O RhD+ red blood cell emergency transfusions (median age 68 years [IQR 55–76]), of whom 85 (20%) patients were later determined to be RhD–. In five patients, blood group samples were not further processed because the patient died soon after admission. 307 (70%) patients were trauma or surgical patients (185 were men and 122 were women); 130 (30%) were medical patients (77 were men and 53 were women; table). Of the 437 patients included in the study, 214 (49%) survived (median age 65 years [51–75]) and 34 (16%) of these patients were RhD–.

Median in-hospital stay was 9 days (IQR 0–25) for RhD+ patients and 7 days (0–18) for RhD– patients; data were missing for 12 RhD+ and six RhD– patients. In-hospital mortality was lower, although not statistically significant, in RhD+ patients (167 [48%] of 347) than in RhD– patients (51 [60%] of 85; OR 1·617, [95% CI 0·998–2·618]; p=0·053). Of the 34 surviving RhD– patients, three were lost to follow-up (figure). The remaining 31 patients were followed up for a median of 12 months (IQR 6–12), and 14 (45%) patients developed anti-D allo-immunisation, with one additional patient shown to be already anti-D positive before emergency transfusion. Only one patient developed anti-D within 4 weeks, whereas in all other

	Patients with unknown blood type transfused with RhD+ red blood cell concentrates (n=437)	RhD– patients transfused with RhD+ red blood cell concentrates due to RhD– red blood cell concentrate shortages (n=110)
Sex		
Female	175 (40%)	26 (24%)
Male	262 (60%)	84 (76%)
Median age (years)	68 (55–76)	66 (58–75)
Underlying cause of bleeding		
Trauma or surgery	307 (70%)	83 (75%)
Medical	130 (30%)	27 (25%)
Red blood cell concentrates transfused		
Until blood group typing	1782 (4, 2–6)	NA
Within 24 h	5152 (8, 4–14)	1474 (10, 6–16)
Within 7 days	5833 (10, 5–16)	1822 (12, 8–20)
Blood transfusions		
1–4 red blood cells units transfused	98 (22%)	12 (11%)
RhD– patients	24 (25%)	12 (100%)
RhD– patients with anti-D allo-immunisation	3 (13%)	4 (33%)
5–8 red blood cells units transfused	99 (23%)	28 (25%)
RhD– patients	17 (17%)	28 (100%)
RhD– patients with anti-D allo-immunisation	5 (29%)	5 (18%)
9–12 red blood cells units transfused	72 (16%)	19 (17%)
RhD– patients	9 (13%)	19 (100%)
RhD– patients with anti-D allo-immunisation	2 (22%)	3 (16%)
13–16 red blood cells units transfused	66 (15%)	16 (15%)
RhD– patients	14 (21%)	16 (100%)
RhD– patients with anti-D allo-immunisation	2 (14%)	3 (19%)
17–20 red blood cells units transfused	24 (5%)	8 (7%)
RhD– patients	4 (17%)	8 (100%)
RhD– patients with anti-D allo-immunisation	0	2 (25%)
>20 red blood cells units transfused	78 (18%)	27 (25%)
RhD– patients	17 (22%)	27 (100%)
RhD– patients with anti-D allo-immunisation	2 (18%)	5 (19%)

Data are n (%), median (IQR), or n (median, IQR). Immunisation rates are given for the RhD– patients. RhD=Rhesus D. NA=not applicable.

***Table*: Patient baseline characteristics**

allo-immunised patients, anti-D became detectable more than 4 weeks after transfusion of RhD+ red blood cell concentrates. No acute or delayed haemolytic transfusion reactions were reported.

In 15 RhD– patients, a positive direct antiglobulin test was observed within the first 4 weeks. In all patients with a positive direct antiglobulin test, an eluate was done. In only one (female, medical patient, aged 85 years) of these 15 patients, anti-D was detected within the first 4 weeks. In two patients, the eluate showed pan-reactive agglutinations (without later formation of anti-D; two male surgical patients, aged 64 and 69 years). Three patients with a positive direct antiglobulin test but an initially negative eluate later developed anti-D allo-immunisation (including the patient with anti-D detection within the first 4 weeks; two male patients and one female cardiac surgery patient, aged 69, 80, and 85 years, respectively). In the remaining ten patients, no red blood cell allo-antibodies were detected (five male and five female patients, six surgical and four medical patients, aged 49–83 years). More than 6 months after the RhD+ red blood cell transfusion we found a positive direct antiglobulin test in seven patients (six male patients and one female patient, five surgical and two medical patients, aged 27, 49, 68, 68, 73, 78, and 80 years). The eluate of their autologous red blood cells showed pan-reactive red blood cell antibodies. In five of these patients anti-D antibodies were also present.

The overall risk of developing anti-D allo-immunisation in the 437 emergency patients transfused with O RhD+ red blood cell concentrates ranged between 14 (3%, 95% CI 1·91–5·30) of 437 (assuming that none of the three patients lost during follow-up developed an anti-D) and 17 (4%, 2·44–6·14) of 437 (assuming that all three patients lost to follow-up developed anti-D; figure).

1782 O RhD+ red blood cell concentrates of the emergency depot were transfused. We then continued to transfuse RhD+ red blood cell concentrates in (the meanwhile typed) RhD– patients during the first 24 h after admission. In total, this saved 2836 RhD– red blood cell concentrates. For comparison, during the 15-year observation period, 21 373 O RhD– red blood cell concentrates were transfused. This shows that we saved a substantial proportion of O RhD– red blood cell concentrates.

During the same observation period, 110 known RhD– patients received 543 RhD– red blood cell concentrates and 1279 RhD+ red blood cell concentrates outside the emergency room, often for intraoperative bleeding during elective surgery in times of shortages of RhD– red blood cell concentrates (table). 48 (44%) of 110 patients died in hospital, and 22 (20%) of 110 patients developed anti-D antibodies. Seven patients were lost to follow-up. The remaining 55 patients were followed up for a median of 12 months (IQR 3–12).

The overall risk of developing anti-D antibodies in the entire population of the 110 RhD– patients transfused with RhD+ red blood cell concentrates due to a shortage of RhD– red blood cell concentrates ranged between 22 (20%, 95% CI 13·6–28·4; assuming that none of the patients lost during follow-up developed anti-D) and 29 (26%, 95% CI 19·0–35·3; assuming that all patients lost during follow-up developed anti-D antibodies; figure). The immunisation rate of the RhD– patient population, which has to be transfused with RhD+ red blood cell concentrates due to shortages of RhD– red blood cell concentrates, was significantly higher than in the emergency patient population (26% *vs* 4%, OR 8·845, 95% CI 4·447–17·717; p<0·0001).

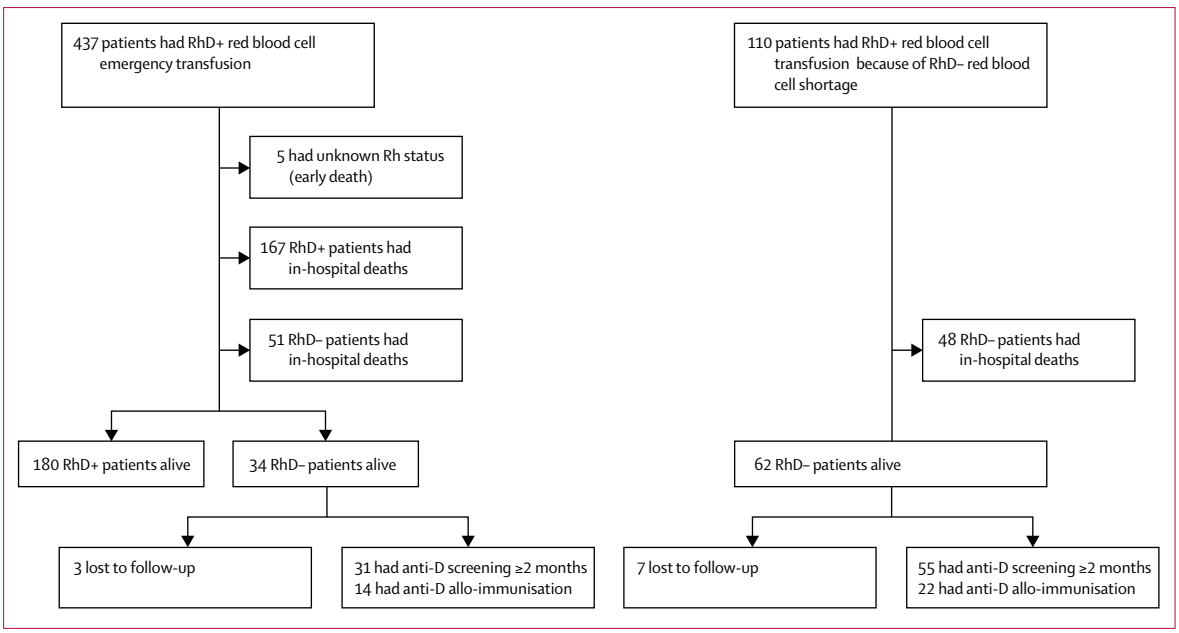

***Figure*: Study profile of enrolled and assessed patients with unknown blood type who received O RhD+ red blood cell concentrates and RhD– patients transfused with RhD+ red blood cell concentrates because of RhD– red blood cell concentrate shortages**
RhD=Rhesus D.

RhD– patients receiving O RhD+ red blood cell concentrates showed a higher mortality than RhD+ patients receiving O RhD+ red blood cell concentrates (60% *vs* 48%; p=0·053) in our study cohort. We assessed the database of another multicentre study on patients requiring massive transfusions.[10] For our post-hoc analysis, 863 patients were assessable for mortality 30 days after massive transfusion. Of the 118 RhD– patients, 43 patients were exclusively transfused with 1344 RhD– red blood cell concentrates and 75 received also 1583 RhD+ red blood cell concentrates. The mortality of the RhD– patients transfused with RhD+ red blood cell concentrates (26 [35%] of 75) did not significantly differ from the mortality of RhD+ patients (282 [38%] of 745; OR 0·871, 95% CI 0·513–1·471; p=0·62). Also, the mortality of RhD– patients receiving exclusively RhD– red blood cell concentrates (15 [35%] of 43) did not significantly differ from the mortality of RhD– patients who also received RhD+ red blood cell concentrates (26 [35%] of 75; OR 0·990, 95% CI 0·419–2·345; p=1·000).

Discussion

This study shows the feasibility of providing O RhD+ red blood cell concentrates for patients with unknown blood type who require urgent red blood cell transfusions. The overall risk of inducing anti-D allo-immunisation by this transfusion strategy was as low as 3–6%. At the same time, the strategy saves at least 10% of the total demand of O RhD– red blood cell concentrates, and reduces the risk of shortages of O RhD– red blood cell concentrates. Prevention of O RhD– red blood cell concentrates shortages reduces the necessity to transfuse known RhD– patients with RhD+ red blood cell concentrates and thereby the overall risk of inducing anti-D in the population. Many physicians are afraid to induce adverse effects by transfusing O RhD+ red blood cell concentrates to patients with unknown blood type. While the risk of inducing acute haemolytic transfusion reactions is low, the risk of inducing anti-D production is considered to be high. However, to determine the overall risk at the time the decision for the emergency transfusion has to be made, the entire population of patients receiving the emergency transfusion needs to be considered, not only the RhD– patients who ultimately survive. This is highly relevant because only 15% of the general population are RhD– and therefore at risk of developing anti-D. The high mortality in emergency transfused patients further reduces the risk of immunisation. Half of the patients will die before the immune response develops. Our strategy to transfuse emergency room patients with O RhD+ red blood cell concentrates saved more than 10% of our overall O RhD– red blood cell concentrate demand. Without these additional O RhD– red blood cell concentrates, it would have been necessary to transfuse a higher number of known RhD– patients with RhD+ red blood cell concentrates, with up to a third of them developing anti-D antibodies. Overall, this would have resulted in a higher number of anti-D positive individuals in our patient population.

We found a numerically higher mortality in RhD– patients receiving RhD+ red blood cell concentrates than in RhD+ patients, which was probably due to chance: when we did a post-hoc analysis of an independent control cohort of massively transfused patients,[10] we

found neither a difference in the 30-day mortality rate between RhD– and RhD+ patients transfused with RhD+ red blood cell concentrates, nor between RhD– patients receiving only RhD– red blood cell concentrates and those also transfused with RhD+ red blood cell concentrates.[10]

Only one RhD– woman was below the age of 46 (aged 39 years) and received O RhD+ red blood cell concentrates. Potentially, younger women are at low risk of emergency transfusion. In the multicentre post-hoc analysis[10] of patients with ultramassive transfusions, only 1·6% were female trauma patients aged younger than 46 years.[10] Given the significant rate of allo-immunisation of RhD– patients and the small number of women of childbearing capacity requiring emergency transfusions, an alternative policy could be to provide women younger than 50 years of age with O RhD– red blood cell concentrates.

During the same observation period, we had to transfuse 110 patients known to be blood group RhD– with RhD+ red blood cell concentrates because of a shortage of RhD– red blood cell concentrates. This number is already higher than the number of the emergency transfused RhD– patients, underscoring the relevance of RhD– red blood cell concentrate shortages. Compared with the group of emergency patients with unknown blood type, the overall risk of this patient group developing anti-D allo-immunisation was about five times higher (20–30%) primarily because all of these patients were RhD– and because a greater proportion of patients survived.

Healthy volunteers systematically and repeatedly immunised with RhD+ red blood cells show an anti-D immunisation rate of about 90%.[13] However, in studies enrolling RhD– patients receiving RhD+ red blood cell concentrates[1–3,11] (not volunteers who are immunised by an immunisation programme) immunisation rates were lower, in the range of 10–30%. Potentially, this lower rate is due to downregulation of the immune system in these critically ill patients. None of the RhD– patients, who received the O RhD+ red blood cell concentrates before the blood group was typed, developed a symptomatic acute or delayed haemolytic transfusion reaction. The patient with anti-D antibodies before emergency transfusion was switched to RhD– red blood cell concentrates as soon as the anti-D antibody was identified and required additional transfusions (eight RhD– red blood cell concentrates in 24 h) because of major bleeding in which he probably lost most RhD+ red blood cells after transfusion.

The low rate of pre-existing anti-D allo-immunisation is consistent with a large Australian study, which found Rhesus allo-antibodies in only 1·5% of 15 966 newly admitted patients.[14] As anti-D prophylaxis in pregnancy is standard since the early 1970s in most developed countries, the risk of pre-immunisation with anti-D in women caused by pregnancy is low.

An allo-immune response can be associated with formation of auto-reactive antibodies, sometimes causing clinically relevant haemolysis several months after transfusion.[15] In our study, seven patients showed a positive direct anti-globulin test with panreactive red blood cell antibodies in the eluate more than 6 months after RhD+ red blood cell transfusion. Among them, five patients had developed an anti-D response, further corroborating that a strong red blood cell allo-immune response can provoke induction of red blood cell auto-antibodies.[15] Thus, induction of red blood cell auto-antibodies has to be considered as a longer-lasting potential adverse effect of the transfusion of RhD+ red blood cell concentrates to RhD– patients. Conversely, the positive direct anti-globulin test during the first weeks after RhD+ red blood cell transfusion in 15 of 85 patients was likely caused by unspecific IgG binding to patient red blood cells because of cofactors related to severe comorbidity. Only one of them developed anti-D antibodies early.

Data regarding the consequences of transfusing O RhD+ red blood cell concentrates to all patients with unknown blood type who require emergency (massive) transfusions are sparse, without prospective randomised trials. Meyer and Uhl[5] reported a retrospective analysis of their transfusion strategy—first transfusing four O RhD– red blood cell concentrates, followed by RhD+ red blood cell concentrates in RhD+ patients or in RhD– patients if not enough RhD– red blood cell concentrates were available.[5] Of 268 patients, 39 (15%) were RhD–, 18 received RhD+ red blood cell concentrates, and only one of them developed anti-D antibodies (0·4% of all patients and 5·6% of RhD– patients). These authors also concluded that the low immunisation rate would justify immediate transfusion of RhD+ red blood cell concentrates in the emergency room. A second retrospective analysis[1] found an anti-D immune response in 16 of 78 RhD– patients receiving RhD+ red blood cell concentrates, with a calculated probability of developing anti-D below 42% (upper 95% confidence bound) and estimated as 30%.[1] A third retrospective analysis[2] of 98 RhD– patients who received a total of 445 RhD+ red blood cell units identified 22 (22%) of 98 patients as developing anti-D antibodies.[2] One prospective study,[11] which analysed the use of RhD+ red blood cell concentrates in 351 RhD– patients also found an incidence of anti-D allo-immunisation in 21% of patients,[11] while the second prospective study[3] found development of anti-D antibodies in 3 (12%) of 26 RhD– patients receiving RhD+ red blood cell concentrates during times of RhD– red blood cell concentrate shortages.[3]

Our study has limitations. Although we collected data prospectively, the long study period of 15 years, as well as the difficult task of obtaining informed consent in this emergency room population before transfusion forced us to design this study as a prospective quality control measure of standard care rather than a randomised trial.

Laboratory parameters typically associated with haemolysis (bilirubin, haptoglobin, free haemoglobin) were not systematically collected, thus subclinical delayed haemolytic reactions might have been missed. We lost several patients to follow-up; therefore we used a conservative analytical approach by assuming that all patients who were lost to follow-up had developed an anti-D response. Although for no patients clinical symptoms of haemolysis after discharge had been reported, we cannot exclude subclinical auto-immune haemolytic anaemia having occurred in some patients. RhD– patients who received most RhD+ red blood cell concentrates had the highest mortality (data not shown). This is due to the typical bias in non-randomised transfusion studies that those patients with the most severe underlying disease typically require most red blood cell transfusions. Our study design does not allow drawing definite conclusions on the number of RhD incompatible transfused red blood cell concentrates and mortality. The strength of our study is that we enrolled all RhD– patients who received RhD+ red blood cell concentrates during the entire observation period, which excludes any enrolment or exclusion bias. Additionally, the endpoints (mortality and development of anti-D antibodies) are objective and not subject to bias.

Taken together, transfusing all patients with unknown blood type who require urgent red blood cell transfusions with O RhD+ red blood cell concentrates will induce an anti-D allo-immunisation in 3–6% of patients. This transfusion policy saves at least 10% of the O RhD– red blood cell concentrate stock and reduces shortages of RhD– red blood cell concentrates. As such, the need to transfuse RhD+ red blood cell concentrates to patients with known RhD– blood type, in whom the risk of inducing anti-D production is much higher (20–30%) will be reduced. In medical systems with shortages of RhD– red blood cell concentrates, transfusing all patients with unknown blood type who require urgent red blood cell transfusions with O RhD+ red blood cell concentrates therefore reduces the overall risk to induce anti-D allo-immunisation in the population.

Contributors

All authors contributed substantially to the manuscript. KS and AG contributed substantially to the study conception and design. GJ, SS, BM, KS, and AG contributed to data acquisition, analysis, or interpretation. KD contributed to acquisition of data and laboratory testing. KS, SS, and AG drafted the Article or critically revised important intellectual content. KS, GJ, KD, SS, BM, and AG approved the final version to be published. All authors agree that they are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the Article are appropriately investigated and resolved. No writing assistance other than copy editing was provided. AG holds a full professorship in transfusion medicine.

Declaration of interests

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For the **BEST collaborative** see http://bestcollaborative.org

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