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Red cell alloimmunisation following intrauterine transfusion and the feasibility of providing extended phenotype-matched red cell units

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SUMMARY

Objectives: To analyse the incidence of additional alloantibody formation following intrauterine red cell transfusion and to evaluate the feasibility of providing extended phenotype-matched red cells in future intrauterine transfusion (IUT).

Background: IUT is performed in severe, life-threatening fetal anaemia, usually in alloimmunised pregnancies. Its complications include the formation of additional alloantibodies to other red cell antigens.

Materials and methods: This was an 11-year retrospective, observational study of additional alloantibody formation in patients receiving IUT in the National Maternity Hospital, Dublin. The study included evaluation of the donor population in the Republic of Ireland (RoI) with regards to the feasibility of providing extended phenotype-matched units in future IUT.

Results: Following IUT, 22% of mothers formed additional red cell alloantibodies. In 67% of cases, the transfused donor red cells expressed the cognate antigen. Suitable donors are available for most combinations of Fy, Jk and Ss antigens.

Conclusions: In our population, it is feasible to provide more extensively phenotype-matched red cells for future IUT. These can be supplied from the current donor pool with no significant extra phenotyping required. We consider their provision to be a reasonable proactive step in a known at-risk group.

Key words: alloantibody, extended phenotype match, haemolytic disease, intrauterine transfusion.

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Red cell alloimmunisation in pregnancy occurs following exposure to foreign red blood cells (RBCs) via transfusion or feto-maternal haemorrhage (FMH), the passage of fetal RBCs across the placenta into the maternal circulation. Low level FMH occurs in up to 85% of pregnancies and is more common later in the gestation period or at delivery (Wylie & D'Alton, 2010). Haemolytic disease of the fetus and newborn (HDFN) develops when fetal RBC antigens stimulate the production of clinically significant maternal IgG antibodies. When these antibodies cross through the placental barrier they can cause destruction of the fetal RBCs or their progenitors. Fetal anaemia can occur by different mechanisms, including suppression of erythropoiesis or extravascular haemolysis.

Intrauterine transfusion (IUT) is the primary treatment of significant fetal anaemia due to the presence of maternal RBC immunoglobulin G (IgG) alloantibodies, and less commonly to parvovirus infection. IUT is an invasive procedure that carries inherent risks (Van Kamp *et al.*, 2005) including the formation of additional RBC alloantibodies (Vietor *et al.*, 1994; Watson *et al.*, 2006; Schonewille *et al.*, 2007). In early pregnancy, the placenta implants randomly and when it implants on the anterior wall of the uterus the route of needle insertion may be transplacental or alternatively transamniotic. The transplacental route has been previously associated with an increased risk of FMH post-IUT and additional alloantibody formation (Schonewille *et al.*, 2007). Additional RBC alloimmunisation due to IUT may further complicate subsequent pregnancies and could delay transfusion if blood is required urgently in future.

The aim of this study was to analyse the level of additional alloimmunisation in IUT recipients in the National Maternity Hospital (NMH), Dublin, Ireland, in an 11-year period. A secondary analysis compared the route and site of needle insertion with incidence of alloimmunisation. We also assessed the current donor population database in the Republic of Ireland (RoI) to ascertain the feasibility of providing extended phenotype-matched units for future IUT recipients.

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MATERIALS AND METHODS

The following specifications apply to RBC units used for IUT in this study: Group O, low titre anti-A/B (donor plasma reactive with A1B cells at a dilution of 1/100 are considered high titre), D-C-E-K- (unless anti-c implicated), <5 days old, Cytomegalovirus (CMV) seronegative, antibody screen negative, indirect antiglobulin test (IAT) compatible with maternal plasma and antigen negative if additional alloantibodies present, volume 190–310 mL, haematocrit 0·7–0·85, haemoglobin ≥40 g/unit, leucocyte content $<1 \times 10^6$/unit, with a 24-h shelf-life post-irradiation. Until November 2010, units for IUT were suspended in citrate phosphate dextrose and adenine (CPDA-1). After November 2010, units have been suspended in citrate phosphate dextrose (CPD). All units were leucodepleted.

The detection of alloantibodies was performed by both the NMH and the Irish Blood Transfusion Service (IBTS) by IAT (gel column or tube) alone or in combination with enzyme-treated cells and/or enzyme/IAT. Donor phenotyping was performed by automated and manual serological methods. Fetal phenotyping was performed on a sample taken before the first IUT for Rh/K and for other antigens where cognate antibodies were present prior to the first IUT, however, it was not standard practice to perform extended phenotype pre-IUT for other antigens. Units for IUT were only matched for antibodies detectable before each IUT during the course of the study period.

The availability of extended phenotyped donors was assessed using search functions on the IBTS laboratory information system (Progesa; MAK-Systems, Paris, France). The following criteria were used to determine suitability for neonatal use: CMV antibody negative, donated within previous 2 years (therefore considered 'regular' donors) but not the last 3 months, previously antibody screen negative and high titre anti-A/B negative. Various combinations of the Fy, Jk and Ss system antigens were assessed, such as O RhD negative C- E- K- Jk^a- Fy^a- S- or O RhD positive E- c- K- s- Jk^b-. The requirement for previous donation in the last 2 years results in variable donor availability on a day-to-day basis. The results presented in this study show donors available on 28 August 2012.

IUT procedures involved infusion of pre-calculated volumes of blood into the sedated fetus under ultrasound guidance. The umbilical cord insertion was the most common site of transfusion followed by the intrahepatic vein (IHV) and free cord loop.

Data was collated in Microsoft Excel 2010 (Redmond, WA, USA) for analysis. Approval for the study was received from the Ethics Committee of the National Maternity Hospital, Holles Street, Dublin, Ireland.

Subjects studied

This retrospective, observational study included all patients in the NMH between 1 January 2002 and 31 December 2012, who received IUT due to fetal anaemia as a result of the presence of red cell alloantibodies. The NMH is the main tertiary referral centre for the treatment of fetal anaemia by IUT in the RoI. Computer databases were accessed at both the NMH and the Red Cell Immunohaematology Laboratory of the IBTS. Patient's files were analysed for the following: additional RBC alloimmunisation and antibody specificities, gestational time of antibody detection, route and site of needle insertion, IUT after which antibody became detectable and antigen phenotype of donor units and fetuses. Fetal antigens were specified for Rh/K and for other known antibodies, but not for antigens other than these.

Patients not antibody screened >3 days post IUT were excluded, as it has been reported that only 0·4% of antibodies are likely to be formed or detected in the first 3 days post-transfusion (Schonewille *et al.*, 2006). Antibody identification was performed both antenatally and post-natally for some patients. Patients receiving multiple IUTs were investigated for additional antibodies prior to subsequent IUT, but not always after the last IUT. These patients were included for the statistical calculation of incidence of patients forming additional antibodies. The calculation of incidence of alloantibody formation after individual IUT did not include IUTs with no subsequent antibody screen.

RESULTS

There were 63 mothers with 71 fetuses requiring IUT in 70 pregnancies (one set of twins) in the 11-year period of this study. A total of 173 units were transfused (median 2, range: 1–5). The primary cause of significant HDFN was attributed to either anti-D, anti-K or anti-c. There were a total of 44 antibodies detectable in 38 of 63 (60%) mothers, before the first IUT, in addition to either anti-D, anti-c or anti-K. These were mainly due to anti-C ($n = 26$), but also anti-E ($n = 6$), anti-G ($n = 4$), anti-Jk^a ($n = 4$), anti-Fy^a ($n = 2$), anti-Jk^b ($n = 1$) and anti-S ($n = 1$).

There were 51 mothers requiring IUT due to anti-D (81%), 8 due to anti-K (13%), 3 due to anti-c (5%) and 1 due to both anti-c + K (1%). Antibody screen was performed >3 days post-IUT in 60 of 63 patients and after 170 of 173 IUTs. Of these patients 13 of 60 (22%) formed a total of 18 additional RBC alloantibodies. The incidence of alloantibody formation after each individual IUT unit is 18 of 170 (11%).

Following donor and fetal phenotype analysis, 5 of 18 (28%) of the additional alloantibodies detected were due to fetal stimulation (Table 1). Fetal phenotype corresponding to additional alloantibodies formed post-IUT, was not known, however, the causative antigen was present on donor RBC in 9 of 18 cases and suspected present in three cases (total 12/18 – 67%). In the three 'suspected' cases, one donor was Fy^a- and the patient formed anti-Fy^b; two donors were S- and the patients made anti-s. In an Irish donor population, donors are highly unlikely to be Fy(a-b-) or S-s-. Neither donor nor fetal antigen status were known in 1 of 18 (6%) cases.

Of the new additional RBCs detected post-IUT, 7 of 18 (39%) were Rh/K-related and 11 of 18 (61%) were non-Rh/K-related. In 5 of 18 (28%) cases additional alloantibodies were detected after the first IUT, 7 of 18 (39%) of alloantibodies were detected after the second IUT and 6 of 18 (33%) after the third, including some that were detected post-natally. Notably a total of 14 of 18

Table 1. Data analysis of additional red cell alloantibody formation post-IUT

Patient	Antibody(s) detectable before first IUT	Additional antibody detected	Week detectable (week + days)	IUT after which antibody became detectable	Donor(s) antigen status
1	D	Fy^b	29	First of 3	Fy^b+
		Jk^b	36	Third of 3	Jk^b+
2	C + D	Jk^b	32	Second of 3	Jk^b+
3	D	C	29 + 4	First of 1	C-[1]
		K	Detected in subsequent pregnancy	First of 1	K-[1]
4	C + D	Jk^b	36	Third of 3	Jk^b+
5	D	E	36 + 1	First of 1	E-[1]
6	D	C	32 + 5	Second of 3	C-[1]
		Jk^a	34 + 4	Third of 3	Jk^a+
		s	36 days PN	Third of 3	S- (s+)[2]
7	C + D	s	9 days PN	Second of 2	S- (s+)[2]
8	C + D	Fy^b	33	Second of 2	Unknown
9	D	C	33 + 1	Second of 3	C-[1]
10	C + D	Fy^b	35 + 4	Second of 2	Fy^a- (Fy^b+)[3]
		S	28 days PN	Second of 2	S+
11	c	Cw	34 + 5	Third of 3	Cw+
12	K	c	34 + 2	First of 1	c+
13	C + D + Jk^a	Fy^b	30	Third of 4	Fy^b+

PN, post-natal.
[1]Antibody was fetal-stimulated.
[2]Donor most likely s+ in Irish donor population.
[3]Donor most likely Fy^b+ in Irish donor population.

(78%) of the alloantibodies formed were detectable within the current pregnancy gestation period.

Of antibodies formed after the first IUT, 3 of 5 (60%) were known to be of fetal origin, as were 2 of 7 (29%) of antibodies formed following the second IUT. There were no antibodies of known fetal origin formed following subsequent IUTs (0/6); most fetal blood is donor-derived at this stage.

The IUT site of entry was known in 159 of 173 cases. The cord insertion was targeted in 151 of 159 (95%) cases, the IHV in 6 of 159 (4%) cases and the free cord loop in 2 of 159 (1%) cases. Transfusion was via the cord insertion for all cases of additional alloantibody formation. The route of insertion was known in 150 of 173 cases and overall 65% (97/150) of needle insertions traversed the placenta. There was more than one transfusion prior to antibody formation in some cases. Where there was additional alloantibody formation and the insertion route was known ($n = 22$), 77% (17/22) were transplacental, which is not significantly different to the overall rate ($P = 0{\cdot}34$).

Extended phenotyped donor availability

The IBTS database search for various combinations of Fy, Jk and Ss antigen negative donors suitable for IUT are shown in Table 2 for O RhD negative and O RhD positive donors. There are large numbers of donors negative for Fy^a, Fy^b, Jk^a, Jk^b and S antigens when required on their own, and there was therefore no requirement to document the numbers in these categories. These antigens were only searched for in combination with other antigens. There are not always s antigen negative donors available, and searches were performed for s antigen negative donors for both O RhD negative and O RhD positive donors. Analysis of the combination searches shows that there are substantial numbers of donors available for most phenotype combinations. The s antigen negative phenotype is the rarest of those searched (approximately 10% in Caucasian population) and there are low numbers of donors when this antigen is required in combination with some Fy/Jk combinations. There were more O RhD negative C- E- K- donors than O RhD positive E- c- K- donors available; however, the majority of IUT donation units required were O RhD negative (94% in this study).

DISCUSSION

Results from this study show that at least 22% of patients undergoing IUT form additional alloantibodies to red cell antigens. This is slightly lower than other studies where rates of 25% (Schonewille *et al.*, 2007) and 26% (Watson *et al.*, 2006) were encountered. In comparison to some patient groups, the majority of pregnant women are healthy with fully functioning immune systems. Alloimmunisation to RBC antigens can take weeks to months, which enables time for cells to age naturally, undergo phagocytosis and removal by the mononuclear phagocyte system. The signalling events created when

Table 2. Availability of O RhD negative (rr) and O RhD positive (R_1R_1) extended phenotyped donors suitable for IUT

Number of donors available	O RhD negative C-E-K- extended types ($n = 5668$)		O RhD positive E-c-K- extended types ($n = 4349$)	
>100	Jk^b- S-	Fy^a- Jk^b-	Jk^b- S-	s-
	Jk^a- S-	Fy^a- Jk^a-	Jk^a- S-	Fy^a- Jk^b-
	Fy^a- S-		Fy^a- S-	Fy^a- Jk^a-
50–100	Fy^a- Jk^b- S-	s-	Fy^a- s-	Fy^b- S-
	Fy^a- Jk^a- S-	Fy^b- S-	Fy^a- Jk^b- S-	Fy^a- Jk^a- S-
10–50	Fy^b- Jk^b-	Jk^b- s-	Jk^b- s-	Fy^b- Jk^b- S-
	Fy^b- Jk^a-	Fy^b- Jk^a- S-	Jk^a- s-	Fy^a- Jk^a- s-
	Fy^a- s-	Fy^a- Jk^b- s-	Fy^b- Jk^a-	Fy^b- s-
	Fy^b- Jk^b- S-	Jk^a- s-	Fy^a- Jk^b- s-	Fy^b- Jk^a- S-
<10	Fy^b- s-	Fy^b- Jk^b- s-	Fy^b- Jk^b- s-	Fy^b- Jk^a- s-
	Fy^a- Jk^a- s-	Fy^b- Jk^a- s-		

Data details donors available on 28 August 2012.

a pathogen is encountered are not necessarily present when RBCs are transfused and the increased speed and rates of alloimmunisation in pregnancy may be in part due to the presence of inflammatory signals (Hendrickson *et al.*, 2006; Kumpel & Manoussaka, 2012), especially given the small volumes associated with FMH. Various commentators have suggested the need to identify potential responders and provide phenotype-matched blood where possible (Higgins & Sloan, 2008; Anstee, 2009). The majority of pregnant women who receive IUTs have already made at least one alloantibody and have therefore already identified themselves as 'responders'.

There are various reasons why IUT is associated with increased alloimmunisation. It is an inherently invasive procedure being performed in an immunologically primed individual. It involves penetration of the fetal blood system with irradiated RBCs administered through a syringe under physical pressure, potentially adding to the fragility of the RBC membrane with possible increased rates of deterioration and clearance. IUT is associated with increased FMH, transmitting both donor and fetal cells into the maternal circulation (Verduin *et al.* 2010). Usually RBC alloantibodies do not affect the current pregnancy as FMH and alloimmunisation tend to occur towards the end of the gestation period. In the IUT recipients in this study, we observed additional alloimmunisation antenatally (78%) and early in the gestation period (<30 weeks in two cases).

In this study 67% of donors were antigen positive for antibodies subsequently formed. Although fetal antigen status was unknown, we now attempt to perform extended phenotype-matching of IUT recipients where feasible, once sufficient is given. Extended matching and donor sourcing must not however impede the delivery of transfusion or compromise patient outcome when clinically urgent. Ideally, maximum notice should be given to the blood centre in order to have suitable units available. In the event that fully phenotype-matched donors are not available, 'best match' blood should be selected. This may be based on donor availability and the immunogenicity of antigens involved. This has been previously recommended by other groups (Watson *et al.*, 2006; Schonewille *et al.*, 2007) and is current policy in Australia and New Zealand (ANZSBT Antenatal Guidelines, 2007) and the Netherlands (Schonewille, 2013 personal communication). In order to do this, aspects of cost and feasibility of such a programme must be considered. Sourcing of donors is required and they may be requested to donate at short notice which can be inconvenient. Routine extended phenotyping of donors can be costly and typing procedures are time-consuming. However, most blood centres in developed countries now have extensive serological antigen typing capacity and the use of high throughput molecular typing methods are also available, resulting in large databases of extended phenotype donors.

Currently the IBTS performs extended phenotypes (other than Rh/K) on approximately 20% of its donors on a routine basis. This amounts to over 30·000 units per year. As the IBTS issues approximately 20 units for IUT per year, this equates to only a very small percentage of units that are extended typed per year (<0·1%). We analysed the availability of extended phenotyped donors that meet the IUT suitability criteria, for various combinations of antigens (Table 2). This study shows that providing extended phenotype-matched donors for most IUT recipients is achievable, given the current donor population and without increasing our current level of routine phenotyping. When a patient is s antigen negative in combination with other Fy/Jk antigens, our potential donors are more limited. However, the scarcity of donors negative for some antigen combinations is offset by the likelihood of encountering patients with these phenotypes. We estimate that we need at least 10 donors of a certain phenotype to ensure the provision of blood for IUT (2–3 IUTs), given the difficulties that can sometimes arise when requesting donors to donate. Considering the potential benefits for mother and fetus, we consider it both achievable and justifiable to provide better matched blood for IUT for these patients, provided it does not unduly delay transfusion.

Accurate fetal phenotype testing is not feasible post-IUT, as a large amount of the fetal blood volume has been replaced. Fetal phenotyping must be performed on a sample taken prior to the first IUT. Although fetal genotyping plays a valuable role in the prediction of risk of HDFN, it has limited value in the selection of blood for IUT. Ideally, donor selection should be based on the antigen(s) not expressed by the mother, regardless of known fetal type. Additional antibody formation has implications for the current and future fetus and could exacerbate the severity of HDFN. The formation of antibodies post-IUT has previously led to difficulties in the IBTS, in sourcing blood at short notice where additional antibodies are detected immediately prior to subsequent IUT. Extended matching will not prevent alloantibody formation due to fetal stimulation nor will it prevent future HDFN due to alloantibodies present pre-IUT. However, we deem it a reasonable proactive step in a

known at-risk group and recommend its consideration in other centres.

We acknowledge some potential limitations to this study. Owing to the lack of extended fetal phenotypes routinely performed before transfusion, we could not state whether alloantibodies are donor- or fetal-stimulated in many cases. This could be addressed in a future prospective study; however, extended matching of donor units for future IUT in the RoI may eliminate this possibility. However, it is notable that 67% of donations used for IUT were antigen positive for the cognate antibodies that were formed. We could not account for patients who may have been 'accidentally' phenotyped matched, as this information was not available. The risk of alloimmunisation could theoretically be under-reported in this regard.

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CONFLICT OF INTEREST

The authors have no competing interests.

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