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A correlation between severe haemolytic disease of the fetus and newborn and maternal ABO blood group

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SUMMARY

Objective: To analyse anti-D quantification levels and frequency of intrauterine transfusion (IUT), per maternal ABO blood group.

Background: Maternally derived red cell allo-antibodies can target fetal red cell antigens *in utero* leading to haemolytic disease and fetal anaemia. When a clinically significant allo-antibody is formed the priority is ascertaining the risk to the fetus and maternal ABO blood groups are not considered relevant.

Materials and methods: This was a 10-year retrospective, observational study carried out on women referred for anti-D quantification ($n = 1106$), and women whose fetuses required an IUT to treat fetal anaemia ($n = 62$) due to anti-D, in the Republic of Ireland.

Results: Relative to the overall incidence of RhD allo-immunisation by blood group, women of blood group A were more likely to require IUT compared with those who were blood group O ($P = 0{\cdot}002$).

Conclusion: It is known that ABO feto-maternal compatibility can influence the incidence and level of red cell allo-antibodies in pregnancy; however, it does not account for the significantly high rate of severe haemolytic disease requiring IUT seen in blood group A women.

Key words: ABO blood group, haemolytic disease, intrauterine transfusion.

Haemolytic disease of the fetus and newborn (HDFN) is caused by maternal red cell allo-antibodies targeting fetal red blood cells. Following stimulation during the current or a previous pregnancy, clinically significant immunoglobulin G (IgG) antibodies can cross the placental barrier and target fetal red cells if the cognate antigen is present. This can lead to destruction of the fetal red cells, or their progenitors, resulting in fetal anaemia. The level of HDFN varies from intrauterine death to a positive direct antiglobulin test at delivery. The RhD antigen is highly immunogenic and anti-D is the most significant antibody associated with HDFN (Urbaniak & Greiss, 2000).

Fetal anaemia can manifest itself through a number of different mechanisms, including extravascular haemolysis or the suppression of erythropoiesis, depending on the specificity of the antibodies present. Intrauterine transfusion (IUT) is the primary treatment for significant fetal anaemia in pre-term pregnancies where delivery is not appropriate. IUT is most commonly performed in cases of severe anaemia caused by anti-D but can also be required in anti-c (Appelman *et al.*, 1990) and anti-K (Vaughan *et al.*, 1994) allo-immunisation. Very rarely, other clinically significant red cell allo-antibodies may cause fetal anaemia severe enough to warrant transfusion.

Current guidelines and policies regarding HDFN due to anti-D are centred on primary prevention through prophylaxis and the appropriate management of allo-immunised pregnancies (Gooch *et al.*, 2007). Routine antenatal prophylaxis is not yet standard in the Republic of Ireland (RoI); as such, allo-immunisation to anti-D remains a significant problem. The current policy involves the administration of prophylactic anti-D following potentially sensitising antenatal events and following delivery of an RhD positive infant; however, the introduction of routine prophylaxis is now recommended (Fitzgerald *et al.*, 2012).

Serial assessment of maternal anti-D levels during pregnancy can help guide clinicians to the risk of HDFN. There are various methods employed for the assessment of anti-D including quantification, titration and some functional assays such as the monocyte-monolayer assay. In the RoI the antibody quantification assay is used, with moderate risk and severe risk of HDFN at anti-D levels of 4–15 and >15 IU mL^{-1}, respectively (Gooch *et al.*, 2007).

IUT is performed to correct fetal anaemia primarily caused by maternal red cell allo-immunisation, less commonly to correct fetal anaemia secondary to parvovirus infection and also to correct fetal thrombocytopenia due to platelet allo-immunisation.

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The ABO blood group of the mother is not generally considered relevant to HDFN once immune anti-D has been formed, as the priority is the presence or absence of the RhD antigen on the fetal red cells and the severity of the fetal anaemia, if the fetus is affected. However, anecdotally we have observed a high rate of blood group A in allo-immunised women whose fetuses required IUT to treat fetal anaemia.

As such, the aim of this study was to (i) review the quantification levels of anti-D per ABO blood group and, (ii) examine the distribution of maternal ABO blood groups where the fetus had suffered from severe HDFN requiring IUT due to anti-D.

MATERIALS AND METHODS

This is a retrospective, observational study of RhD allo-immunisation in the RoI over the 10-year study period, July 2002 to August 2012. The Red Cell Immunohaematology (RCI) Laboratory in the Irish Blood Transfusion Service (IBTS) routinely performs anti-D quantification for all antenatal patients in the RoI. Antibody quantification was performed by continuous flow autoanalyser using bromelised CDe/CDe cells (R_1R_1) as described by Marsh *et al.* (1968) and is measured in IU mL^{-1}.

This study analysed ABO blood group distribution and antenatal quantification levels using the highest antenatal level detected per patient. Anti-D levels >1 IU mL^{-1} are generally considered to be immune in nature unless large doses of prophylaxis have been administered (Gooch *et al.*, 2007). While this threshold has been reduced to 0·4 IU mL^{-1} in the most recent guidelines (Qureshi *et al.*, 2014), all women with anti-D levels <1 IU mL^{-1} were excluded in this study, to avoid erroneous inclusion of cases of passive anti-D secondary to prophylactic administration. Quantification values where antibody identification was not performed or was inconclusive were excluded, as were those combined with additional enzyme/auto-antibodies which may have resulted in artificially elevated quantification levels. In women undergoing IUT, only pre-procedure anti-D levels were included, as the transfusion procedure itself can increase antibody levels (unpublished data). Women with additional allo-antibodies present were not excluded.

All statistical analysis involving anti-D quantification data was based on patients with levels >1 IU mL^{-1}. Subgroups of patients with levels between 1–4 and >4 IU mL^{-1}, between 4–15 and >15 IU mL^{-1} were also analysed, using the thresholds recommended by the British Committee for Standards in Haematology (BCSH) (Gooch *et al.*, 2007).

The indication for IUT to treat fetal anaemia was based on Doppler ultrasound, demonstrating that the speed of blood flow in the middle cerebral artery was greater than 1·5 multiples of the median for that gestation (Mari *et al.*, 2000).

The primary aim was to examine the relationship between maternal ABO status and RhD antibody level in allo-immunised pregnancies. The secondary outcome was analysis of the distribution of ABO blood groups of women who required IUT in the RoI due to the presence of anti-D, in the same time period. Statistical calculations involved χ^2 analysis of association for parametric data and Mann–Whitney rank sum for non-parametric data. Collected data was entered into Microsoft Excel 2010 (Redmond, WA) and then transferred into IBM SPSS statistics software version 19·0. Ethical approval for the study was received from the ethics committee of the National Maternity Hospital, Holles Street, Dublin, Ireland.

RESULTS

Between 2002 and 2012, 1106 pregnant women in the RoI provided samples for maternal anti-D quantification which form the basis of this study of a total of 5823 samples. Of these 491 (44%) women had anti-D quantification values >1 IU mL^{-1}. Corresponding maternal blood groups were available for analysis in all cases.

Analysis of anti-D quantification levels and maternal ABO distribution

Among RhD allo-immunised women, women of blood group A ($n = 177$) demonstrated significantly higher maternal anti-D antibody levels compared with blood group O ($n = 224$) (median 12·6 vs 7·59 IU mL^{-1}, respectively; Table 1, Mann–Whitney $P = 0·036$). While there is an increased proportion of blood group A women in higher risk categories, there is no difference in medians between A and O (median 18·11 vs 17·39 IU mL^{-1}, respectively, Mann–Whitney $P = 0·64$) when we compare only those >4 IU mL^{-1}. Women of blood group B had a slightly higher maternal antibody level (median 8·22 IU mL^{-1}) than women of blood group O; however, they were not significantly higher (Mann–Whitney $P = 0·57$). There was no significant difference between the quantification levels between blood group A and B (Mann–Whitney $P = 0·26$). Women of blood group AB had the highest median quantification levels (median 13·54 IU mL^{-1}); however, the sample numbers in this group ($n = 18$) were relatively small for meaningful statistical comparison. ABO distribution using risk categories for HDFN in RhD allo-immunisation recommended by the BCSH Guidelines (Gooch *et al.*, 2007) was also examined (Fig. 1). The proportion of blood group A women significantly increases across the higher risk categories of anti-D when compared with blood group O (Pearson χ^2, $P = 0·039$) as demonstrated in Fig. 1. Despite this there are still less blood group A women in all of the risk categories than O, due to the higher proportion of blood group O in the general population.

Analysis of ABO distribution in pregnancies requiring IUT for RhD allo-immunisation

Over the 10-year study period, 62 women required IUT procedures for fetal anaemia secondary to RhD allo-immunisation. Maternal anti-D levels were >4 IU mL^{-1} in all cases. Blood groups of women undergoing IUT were A (52%, 32/62), O (29%, 18/62), B (16%, 10/62) and AB (3%, 2/62).

We found that women of blood group A were over-represented in the subpopulation of pregnancies requiring

Table 1. Comparison of anti-D quantification levels between blood groups O, A, B and AB

	Blood group O	Blood group A	Blood group B	Blood group AB
>1 IU mL^{-1} median (IQR)	7·59 (2·94 – 23·10) (n = 224)	12·64 (3·87 – 29·47) (n = 177)	8·22 (3·74 – 24·7) (n = 72)	13·54 (3·05 – 38·63) (n = 18)
>4 IU mL^{-1} median (IQR)	17·39 (9·69 – 35·0) (n = 140)	18·11 (10·67 – 35·04) (n = 132)	18·14 (6·55 – 43·98) (n = 52)	18·58 (8·07 – 58·98) (n = 53)

IQR, inter-quartile range.

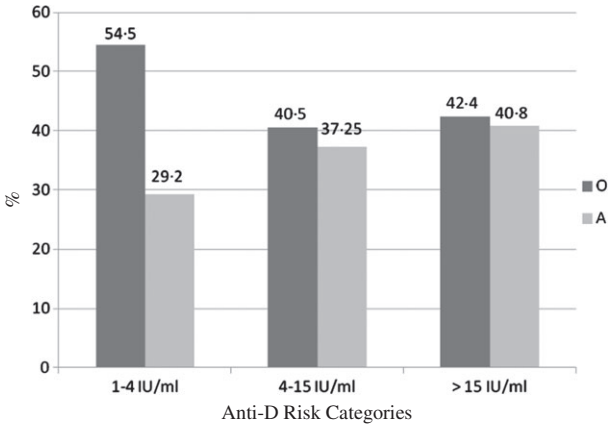

Fig. 1. Distribution of anti-D quantification levels as per BCSH risk categories between blood group O and A. The proportion of blood group A increases significantly compared with blood group O in the higher risk categories (Pearsons χ^2 P = 0·039).

Table 2. Comparison of frequency of blood group O and A RhD negative women requiring IUT

	>1 IU mL^{-1} (n = 401)		>4 IU mL^{-1} (n = 272)	
	IUT	No IUT	IUT	No IUT
O	18	206	18	122
A	32	145	32	100
χ^2 (1 df)	P = 0·002		P = 0·015	

IUT. Specifically, blood group A accounts for 36% of RhD allo-immunised patients but 52% of mothers requiring IUT. Blood group O, however, which accounts for 45·6% of RhD allo-immunised patients, accounted for only 29% of mothers requiring IUT (A vs O, χ^2, 1 degree of freedom (df), P = 0·002, Table 2). The distribution of fetal ABO blood groups recorded in 62 pregnancies, were as expected for A and O.

In the >4 IU mL^{-1} subgroup there was a continued significant difference in the proportion of blood group A and group O mothers who required IUT compared with the incidence of these blood groups in RhD allo-immunised women (χ^2, 1 df, P = 0·015, Table 2). Interestingly, there was also a higher percentage of blood group A mothers with anti-D quantification levels less than 15 IU mL^{-1} (5/32 – 16%), requiring IUT, compared with blood group O (1/18 – 5%).

There was no statistically significant difference found between the proportion of IUT required between blood group B and blood group AB compared with blood group O (χ^2, 1 df, P = 0·18 and P = 0·70, respectively); however, again the sample numbers in these categories were relatively small for statistical analysis.

DISCUSSION

Initial observations regarding the incidence of significant HDFN and maternal ABO blood group indicated a high number of blood group A patients. We analysed a subgroup of patients requiring IUT due to anti-D in a 10-year time period, vs the incidence of immune anti-D in the same time period per ABO blood group. The RCI Laboratory, IBTS is the sole referral laboratory for anti-D quantification and this study includes all recipients of IUT due to anti-D, in the RoI. There is a significant difference between the incidence of women allo-immunised to RhD and those requiring IUT, with regards to maternal blood group A and O (P = 0·002). Blood group O women appear to have a lower risk of carrying a fetus with significant HDFN requiring IUT compared with blood group A and there is a significantly lower proportion of blood group O individuals with quantification levels >4 IU mL^{-1} when compared with blood group A (P = 0·039, Fig. 1).

Blood group O has historically been linked with a reduced likelihood of allo-immunisation in pregnancy. The decrease in incidence of RhD allo-immunisation in group O women is attributed to the 'natural' prophylactic effect of anti-A/B in their plasma. Feto-maternal ABO incompatibility can confer protection against immunisation as the presence of anti-A/B in the plasma of pregnant women facilitates the clearance of A/B fetal red cells post feto-maternal haemorrhage, before immunisation to the RhD antigen can take place. Indeed it was this phenomenon that instigated the idea of anti-D prophylaxis in the late 1950s after Levine initially noticed a lower rate of immunisation to the RhD antigen in ABO incompatible pregnancies (Levine, 1943). Blood group O individuals are more likely to have an ABO incompatible pregnancy and the incidence of allo-immunisation to RhD appears less in blood group O women in the RoI. In a sample of 10 491 RhD negative patients in the National Maternity Hospital, Dublin, the frequency of blood group O was 50·6% (unpublished data), which is higher than the frequency of group O in RhD allo-immunised women (45·6%). The frequency of group A in the same patient sample was 33·6%, which is lower than the frequency of group A in RhD allo-immunised women (36%).

Table 3. Distribution of ABO phenotypes in, women allo-immunised to RhD, fetuses and women requiring IUT due to anti-D, between 29 July 2002 and 1 August 2012

ABO blood group	Women with anti-D >1 IU mL^{-1} (n = 491)	Women with anti-D >4 IU mL^{-1} (n = 337)	Women whose fetus required IUT due to anti-D (n = 62)	Fetuses requiring IUT due to anti-D (n = 62)
O	45·6% (224)	41·5% (140)	29% (18)	56% (35)
A	36·0% (177)	39·2% (132)	52% (32)	34% (21)
B	14·7% (72)	15·4% (52)	16% (10)	6% (4)
AB	3·7% (18)	3·9% (13)	3% (2)	3% (2)

Vos (1965) reported that higher level antibody titres were more likely in ABO compatible pregnancies and this is the case today despite use of anti-D prophylaxis. Blood group A, B and AB all had higher median quantification levels than blood group O.

However, despite any decrease in the incidence or quantification level of anti-D due to natural prophylaxis, blood group O still accounts for more patients with anti-D in all of the risk categories (Table 3) and critically, the incidence does not correlate with the rate of IUT observed per maternal ABO blood group.

ABO incompatibility is also relevant in cases of ABO HDFN whereby the mother has IgG anti-A/B. This form of HDFN is not typically severe (Klein & Anstee, 2005), is usually associated with group O mothers rather than group A or B and is not relevant to the increased incidence of IUT in group A individuals in this study.

ABO blood groups have been associated with various diseases including susceptibility to arterial and venous thromboembolism (O'Donnell & Laffan, 2001), gastric cancer (Aird *et al.*, 1953), malaria (Oliver-Gonialei & Torregroaa, 1944) and ischaemic heart disease (Whincup *et al.*, 1990). Interestingly a correlation between ABO genotype and severe neonatal allo-immune thrombocytopenia (NAIT) has recently been reported (Ahlen *et al.*, 2012) and an increased risk of NAIT was also observed in blood group A mothers. In this study the authors postulated linkage between the maternal ABO genotype and genetic regulation of immune responses, with certain ABO genotypes having a possible influence on gene(s) encoding regulatory factors.

Our data suggests that the ABO blood group of the mother appears to influence the risk of severe haemolytic disease in the fetus. Natural prophylaxis may go some way to explain a decreased rate of allo-immunisation to RhD in blood group O patients; however, it cannot explain the significantly high proportion of IUTs required by blood group A mothers in this study. When assessing the risk of HDFN based on antibody levels, ABO blood group may be an additional factor to consider.

We acknowledge some potential limitations to this study. As this was a retrospective study we were unable to perform any additional testing or investigations on any of the patients. Parity, which can have an effect on antibody levels, was not included in this study as this information was not always available. However, anti-D sample sizes (>1 IU mL^{-1}, n = 491) were deemed sufficient to counteract any possible skewing effect of parity, especially between blood groups A (n = 177) and O (n = 224). The sample sizes for maternal blood groups B and AB requiring IUT were small for meaningful statistical analysis. It would be beneficial to ascertain if the same correlation with regards to ABO blood groups is seen in less severe forms of HDFN.

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CONFLICT OF INTEREST

The authors have no competing interests.

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